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CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

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SOME RELATIONS OF CERTAIN HIGHER PLANTS TO THE FORMATION OF NITRATES IN SOILS

BY T. LYTTLETON LYON AND JAMES A. BIZZELL

OF THE DEPARTMENT OF SOIL TECHNOLOGY

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SOME RELATIONS OF CERTAIN HIGHER PLANTS TO THE FORMATION OF NITRATES IN SOILS

SOME RELATIONS OF CERTAIN HIGHER PLANTS TO THE FORMATION OF NITRATES IN SOILS

T. LYTTLETON LYON AND JAMES A. BIZZELL

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INTRODUCTION

There are several forms in which nitrogen occurs in soils. The uncombined nitrogen of the soil air constitutes the largest supply because of its diffusibility with the atmospheric air. Next in amount is the nitrogen of organic compounds, ranging from .05 to .3 per cent on ordinary arable land and always slightly, but appreciably, soluble in soil water. In upland cultivated soils the nitrogen of nitrate salts forms the next largest supply, but rarely exceeds 20 per cent of the total combined nitrogen of the soil. Curiously enough, the heavy, and consequently poorly aerated, soils usually contain more nitrates than do lighter soils, and Fischer (1911)* has found that nitrate formation is more rapid in such soils. In forest, swamp, and inundated soils the nitrogen of ammonium salts and nitrites forms a larger proportion of the soil nitrogen than does the nitrate nitrogen, but in well-aerated soil these compounds exist in very small quantities.

The utilization of nitrogen in these various forms by agricultural plants has furnished the incentive for a large amount of able and important experimentation, which has resulted in the definite and final demonstration of certain facts and in the partial solution of some questions relating to the agricultural availability of nitrogen in its various soil conditions. The utilization of atmospheric nitrogen by leguminous plants, for instance, has been established beyond question; but the extent to which this form of nitrogen may be utilized by other plants, or the identity of the plants that participate in its use, are subjects on which opinions differ and which are still being investigated. The study of the degree to which the nitrogen of organic compounds is available to higher plants has enlisted the efforts of numerous investigators and has been only partially worked out.

While nitrate nitrogen is generally conceded to be the form best suited to the use of most higher plants, there is undoubtedly a difference between plants of different species in their relation to the nitrate nitrogen content of the soil. The disappearance of nitrate nitrogen from the soil during the growth of different kinds of plants is not always proportional to the total quantity of nitrogen removed by the crop. Thus, it is greater for oats than for maize, although the latter crop may contain a much larger quantity of nitrogen. Both the question of a possible influence of certain plants on the process of nitrate formation in the soil, and that of the degree to which some plants may use forms of nitrogen other than nitrates, are raised by these phenomena.

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* Dates in parenthesis refer to bibliography.

Boussingault first demonstrated the importance of nitrates for higher plants. Previous to that time ammonia had been considered the chief source of nitrogen, and still earlier humus had been considered the source. Liebig gave the weight of his influence in favor of ammonia as the supply. He was unaware, of course, of the transformation of ammonia nitrogen into nitrates in the soil. Since the publication of the experiments by Boussingault and the later work on nitrification, there has been a tendency to consider nitrate nitrogen as the only available supply of nitrogen for autotrophic plants.

It is questionable whether nitrates are so universally and decidedly important to plant growth as has been commonly supposed since the time of Boussingault. The preference that swamp rice plants exhibit for ammonium salts, as proved by Kellner (1884) and also by Kelley (1911), indicates an adaptation of this variety to the prevailing condition of its environment, which must be reversed on its transfer to upland soils. Other plants also may adapt themselves to the use of the more abundant form of nitrogen.

Ammonium salts are toxic to most higher plants except in very dilute solutions. Mazé (1900) obtained as great a growth of maize with a very dilute solution of ammonium sulfate as with a correspondingly strong solution of nitrate. It is possible that the toxic action has masked the nutritive value of ammonia in many experiments.

In a similar way results of experiments with ammonium salts applied to soils may have been influenced by the acid residue remaining when the nitrogen was removed.

Hutchinson and Miller (1911) found that peas obtained nitrogen from ammonium salts as readily as they did from sodium nitrate, but that wheat plants, although able to obtain nitrogen directly from ammonium salts, grew much better in a solution containing nitrates.

One feature brought out by the numerous experiments with ammonium salts is the difference between plants of various kinds in respect to their ability to absorb ammonium compounds. When fully worked out, the degree to which this aptitude is possessed may serve as a practical guide in the fertilizing of agricultural plants.

One of the early beliefs in regard to plant nutrition was that organic matter as such was directly absorbed by higher plants. This opinion was afterwards entirely replaced by the mineral theory propounded by Liebig; and still later the discovery of the nitrifying process almost disposed completely of the belief that organic nitrogen was a food for higher plants. Possibly plants of different species vary in their ability to use nitrogen in the different organic forms. It is also quite likely that the stage of growth of the plant determines, in some measure, the ability of the plant to utilize organic forms of nitrogen.

The relation that the various nitrogenous compounds of the soil bear to the physiological processes concerned in absorption is an almost untouched subject. It is possible that changes occur at the root surface through the action of enzymes secreted by the plant. Nitrates have been found in plants, and so also has ammonia. It is generally held that these substances are absorbed directly.

Nitrate-reducing enzymes have been found in several species of plants, and it seems probable that reduction is the first step in the assimilation of nitrates. Ritter (1912) found that the first process in the utilization of nitrates by certain molds is a reduction of nitrate to nitrite. If amids are an intermediate step in the formation of proteids in the plant, reduction is necessary.

It may be that the formation of nitrates is merely a process that facilitates the absorption of nitrogen by reason of the ease with which nitrates pass through the semipermeable membrane of the root cells, and that the most economical use of soil nitrogen would not necessitate the formation of large quantities of nitrates for the growth of many of the higher plants.

EXPERIMENTS BY OTHER INVESTIGATORS

Experiments by the writers of this paper and by a number of other investigators indicate that crops differ from one another in respect to the occurrence of nitrates in the soil on which they grow. For any one soil the nitrate content is largely dependent on the kind of crop grown. Furthermore, the stage of growth of any particular plant has a characteristic relation to the nitrate content of the soil. The extensive literature bearing on the nitrate content of soils contains a number of instances illustrating this relationship between the soil and the crop growing on it, and these may be so classified as to bring out definite relations.

Experiments showing different nitrate contents of soils under different crops

In an extensive series of field experiments in which nitrates were determined in samples of soil taken under several crops, King and Whitson (1901) found that, for the four crops named, nitrates were highest during the growing season under maize, next under potatoes, and lowest under alfalfa and clover.

Stewart and Greaves (1912), in another extensive set of experiments extending over several years, found the nitrate content under maize, potatoes, oats, and alfalfa to rank in the order named.

In field soil planted to different crops Wollny (1897) found that the quantities of nitric nitrogen, expressed in kilograms per German acre, were 9.2 under beans, 22 in fallow, and 1.2 under wheat.

Brown (1912) sampled soil of neighboring plats that continuously for several years had grown maize and clover, respectively, and found that the processes of ammonification and nitrification were more rapid in the soil planted to maize.

Voorhees, Lipman, and Brown (1907) inoculated a modified Omelianski's solution with soil that had grown oats, soil that had grown crimson clover, and soil that remained unplanted during the period required to mature the crops. The resulting formation of nitrates was greater in the solutions inoculated with the unplanted soil than in those inoculated with the soil planted to either of the crops. The quantity of nitrates found, however, was small. An ammonification test resulted in a greater production of ammonia from the planted than from the unplanted soil.

Brown and MacIntire (1910) found the average nitrate content for the growing season to be: under maize, 55.5 parts per million of the dry weight of the soil; under oats, 18 parts per million; under wheat, 7.9 parts per million; under grass, 1.4 part per million. The average moisture content of the soil under these crops ranged from 24.1 per cent for maize to 26.6 per cent for wheat. The difference in nitrate content, therefore, could not be attributed to differences in soil moisture.

In all these experiments nitrates reached their lowest point under grass before they did under wheat or oats, and under wheat and oats before they did under maize; but even after the minimum nitrate content was reached under all crops they stood in the same order. As these experiments represent soils in Germany, New Jersey, Pennsylvania, Wisconsin, and Utah, thus ranging from a humid to an arid region, the agreement in the relation of the nitrogen content of the soil to the plants used in the experiments is certainly significant.

Experiments showing higher nitrate content under plants than in fallow land

The figures given by Stewart and Greaves (1909), in a study of nitrates in irrigated soils planted to maize, potatoes, and alfalfa, and also on land fallow during a period of three years and planted to oats one year, show nitrates to be higher under maize than in fallow land at certain stages in the growth of the crop, and in some cases the same was true with potatoes. The highest nitrate content under these crops occurred at about the time of their most active growth and in the first foot or the first two feet of soil.

King and Whitson (1902) report an experiment in which one of two contiguous plats of oats had a crop entirely removed on June 20, on which date determinations of nitrates were made in the soil of both plats to a depth of four feet. The crop on the harvested plat was weighed and the dry matter determined. At the end of nineteen days nitrates were again determined in both plats, the remaining oats were harvested and weighed, and the dry matter was determined. No determinations of nitrogen were made with the crop, but the gain in nitrogen for the nineteen days was calculated from the dry weight on the basis of average analyses for oat plants at that stage. The gain in nitrogen in the oat plants which had been growing for nineteen days, minus the loss of nitrates during that time, was twice as great as could be accounted for by the gain of nitrates on the bare plat. No account was taken of the nitrogen in the plant roots, which would make a still greater difference. Evidently there was a much larger supply of available nitrogen in the soil growing oats than was represented by the nitric nitrogen of the bare plat, and this supply was due either to a more active nitrate formation in the soil growing oats or to the utilization of some form of nitrogen other than nitrates. The investigators did not think that a decomposition of nitrates could account for it.

Similar results with clover were obtained by the same investigators, but since clover is a legume this may be accounted for by processes already understood.

Results reported by Jensen (1910) showed that soil planted to maize contained more nitrates during the first part of July than did fallow land.

Fraps (1908) found that fifty to one hundred per cent more nitrogen was removed by maize plants in the first nine weeks of their growth than was apparently transformed from organic compounds into ammonium and nitrate salts in the same time.

Bower (1912) determined nitrates in soil of unplanted plats, both cultivated and uncultivated, and in maize plats cultivated and uncultivated. In both the cultivated and the uncultivated plats nitrates under the maize were higher during July than in the bare soil.

In the experiments cited showing a higher nitrate content of soil under maize than in fallow land, this condition obtained during the period of active growth and usually before full bloom. The experiments in which an unaccountable nitrogen supply was indicated also relate to the earlier stages of growth. They agree in suggesting an activity in nitrate formation under certain plants during early growth which does not obtain in unplanted soil.

Experiments indicating a depressed nitrate formation under plants

Brown and MacIntire (1910) found that the nitrates in oat land did not increase after the crop began to mature until after plowing in the fall. The plowing was done on August 20.

Leather (1912) reports experiments in which large tanks containing soil were planted or fallowed and nitrate nitrogen was determined in the drainage water. Of these gauges, numbers 1 and 3 were cropped and numbers 2 and 4 were fallowed. In 1909 the rainfall was sufficient to replace entirely the water in the gauges. The nitrogen in the crops of 1908 and 1909 plus the nitrogen in the drainage water for that period should be, Leather thinks, equal to the nitrogen in the drainage water of the fallow gauges. He shows by the following figures, however, that this is not the case:

Gauge No.	1	2	3	4
	Pounds per acre			
Nitric nitrogen in drainage water	+261.5	+51.1	+209.6	+53.9
Nitrogen in manure used	......	—50.0	......	—50.0
Nitrogen in maize crop, 1908	......	+49.0	......	+44.7
Nitrogen in wheat crop, 1908–1909	......	+ 7.9	......	+ 7.9
Nitrogen in maize crop, 1909	......	+38.6	......	+31.2
Nitrogen estimated to have been in roots of the three crops	......	+31.8	......	+27.9
Net total	+261.5	+128.4	+209.6	+115.6

It thus appears that there is a smaller quantity of soluble nitrogen in the cropped than in the fallow gauges, and that, either directly or indirectly, the presence of the higher plant prevented the formation of nitrates.

At Grignon it was found by Dehérain (1902), as the result of experiments with large vegetative tanks, that the loss of nitrogen in drainage water from fallow soil amounted to 200 kilograms per hectare; from soil planted to maize, harvested before maturity, the removal of nitrogen by the crop plus that in the drainage water amounted to slightly less than that in the drainage water from the fallow soil; from soil planted to oats the nitrogen in the crop and in the drainage water amounted to only 63 kilograms per hectare; and from soil continuously in grass the drainage water contained almost no nitrogen.

Dehérain attributes this to the drying out of the soil by the growing plants during the season most favorable to nitrate formation, except in the case of maize, which was planted later. Leather (1912), who obtained somewhat similar results, does not accept this explanation.

The writers of this paper (1911) advanced the hypothesis that certain higher plants exert a stimulating or a depressing influence on the process of nitrate formation, depending on the stage of growth. Most of the experiments cited above were performed before that time, and, since no suggestion had previously been made as to any relation between higher plants and nitrate formation, many data that might have thrown light on that subject have probably been omitted from the publications. However, Leather, who published his results in 1912, has considered his data with regard to this hypothesis and expresses himself as considering it a possible explanation for the results obtained by Dehérain and by himself.

INVESTIGATIONS AT THE CORNELL UNIVERSITY AGRICULTURAL EXPERIMENT STATION

In connection with a study of the nitrogen relations of the timothy plant, determinations of nitrates were made during the summers of 1907, 1908, and 1909, in samples of soil taken from field plats under timothy, maize, and oats in rotation. The results of these experiments showed such characteristic relations of the respective plants to the nitrate content of the soil that the study has now been continued for six years. The present paper deals mainly with the field experiments in the investigation.

There were two conditions of the experiments which probably contributed largely to making the relationship obvious. These were (1) the heavy clay soil, which made the movement of nitrates very slow and hence emphasized the relationship between the plant at any particular stage of growth and the nitrate content of the soil; (2) the division of each plat into sections, of which one was always kept bare but received the same soil treatment as the planted sections of the plat and thus permitted a constant comparison of the nitrates in the planted and the unplanted sections.

Experiments on plats manured for timothy

Experiment plats one tenth acre in size were used in this experiment. They were in a rotation of timothy three years and maize, oats, wheat, each one year. Most of them had been fertilized in different ways for timothy, but received no fertilizer for the grain crops that followed. Fertilizers were applied to the timothy in 1905, 1906, and 1907. Manure was applied to the timothy in 1905 and 1907. There were twenty-two plats in all. Their treatment is shown in Table 1:

TABLE 1. FERTILIZER TREATMENT (PER ACRE) OF PLATS MANURED FOR TIMOTHY

Plat	Treatment	Plat	Treatment
711	No fertilizer	717	No fertilizer
712	320 pounds rock superphosphate	718	320 pounds rock superphosphate 80 pounds muriate of potash
713	80 pounds muriate of potash	719	160 pounds nitrate of soda 80 pounds muriate of potash
714	No fertilizer	720	No fertilizer
715	160 pounds nitrate of soda	721	160 pounds nitrate of soda 80 pounds muriate of potash 320 pounds rock superphosphate
716	320 pounds rock superphosphate 160 pounds nitrate of soda		

TABLE 1 (*continued*)

Plat	Treatment	Plat	Treatment
722	160 pounds nitrate of soda 80 pounds muriate of potash 640 pounds rock superphosphate	727	320 pounds nitrate of soda 80 pounds muriate of potash 320 pounds rock superphosphate
723	No fertilizer	728	640 pounds nitrate of soda 80 pounds muriate of potash 320 pounds rock superphosphate
724	320 pounds nitrate of soda 80 pounds muriate of potash 640 pounds rock superphosphate	729	No fertilizer
725	320 pounds nitrate of soda 80 pounds muriate of potash 320 pounds rock superphosphate	730	No fertilizer
		731	10 tons manure, 1905, 1907
726	No fertilizer	732	20 tons manure, 1905, 1907

On these plats samples of soil were taken, to a depth of eight inches, at intervals during the growing season and after the timothy and oats had been removed. The entire twenty-two plats were sampled on the same day or on two consecutive days. The samples were removed from the earth by means of a soil auger. Twelve borings were removed from each plat and thoroughly mixed on an oilcloth surface, and a pint sample was placed in a glass jar. The sample was brought to the laboratory and moisture and nitrates were determined -- the former by drying to constant weight at the temperature of boiling water; the latter by the phenol-disulfonic-acid method, comparisons with a standard nitrate solution being made by means of the colorimeter designed by Schreiner.

In 1908 and 1909, when the plats were planted to maize and oats, respectively, an unplanted space was left for twenty-five feet on the south end of each plat. This unplanted space was cultivated when the plats were in maize, receiving the same cultivation as did the planted part of the plat. When the plats were in oats the unplanted space was kept free from weeds by scraping with a hoe. Soil samples were taken from the planted and the unplanted sections of each plat on the same dates, which permits of a comparison of the effect of a certain soil treatment on the uncropped as well as on the cropped soil. It also serves to show the relation of the crop to the nitrates in the soil, which is a very important factor in their occurrence.

In Table 2 is given a statement of the nitrate content of the planted part of each plat on the dates sampled, during the three years. At the foot

TABLE 2. NITRATES IN SOIL IN PLATS 711 TO 732 AT CERTAIN PERIODS DURING THREE YEARS
(PARTS PER MILLION)

Plat	Timothy, 1907										Maize, 1908						Oats, 1909			
	April 23	May 3	May 14	May 22	May 24	June 6	June 13	June 20	July 24	August 14	May 19	June 22	July 6	July 27	August 10	September 17	April 22	June 24	July 12	August 7
711 c*	4	5	1	2	3	3	5	4	5	7	25	40	62	182	162	...	94	38	8	10
712	5	3	5	3	4	2	2	1	6	6	10	31	53	172	162	...	61	14	7	3
713	11	3	4	3	5	3	3	1	6	8	14	40	59	172	201	...	44	25	9	10
714 c	16	5	7	2	7	4	4	2	6	10	22	52	90	268	238	...	60	18	7	8
715	15	15	6	3	8	2	2	2	5	4	26	57	60	211	158	...	56	10	8	11
716	13	9	8	2	3	3	2	3	5	8	33	69	47	261	156	...	32	5	6	8
717 c	11	2	4	2	2	2	3	4	5	6	20	43	39	211	234	...	45	16	3	2
718	7	1	3	1	2	1	2	2	3	4	12	36	40	224	141	...	57	3	2	2
719	6	3	10	1	2	1	1	1	2	4	21	30	30	178	160	...	66	19	2	3
720 c	8	2	3	2	3	2	2	1	3	6	17	31	102	211	136	...	50	13	2	3
721	5	9	73	22	2	2	1	1	3	5	20	38	66	130	152	...	36	3	4	4
722	3	6	8	2	2	1	1	1	2	5	12	32	47	97	139	...	26	4	2	3
723 c	6	4	5	1	3	1	1	1	3	2	11	47	45	141	198	...	40	4	4	1
724	5	14	14	5	4	2	1	1	2	4	12	36	50	208	117	...	30	4	4	2
725	10	34	37	4	3	2	2	2	9	7	21	50	56	194	106	...	30	3	5	1
726 c	..	..	..	..	..	..	..	..	..	..	11	38	51	228	147	...	27	5	2	1
727	..	..	..	..	..	..	..	..	..	..	15	49	75	194	172	32	41	3	3	1
728	12	52	97	47	39	8	50	8	6	8	15	42	81	178	165	...	59	4	6	2
729 c	..	..	..	..	..	..	..	..	..	..	4	25	101	158	178	49	45	21	3	1
730 c	8	4	3	2	3	2	1	1	2	2	7	27	50	150	152	...	44	15	3	1
731	8	1	4	2	3	3	1	2	2	2	14	26	70	198	178	...	49	9	4	1
732	21	5	4	3	3	2	1	3	3	3	44	64	106	251	185	75	63	10	6	3
Average	9	9	16	6	5	2	4	2	4	5	18	41	63	192	165	52	48	11	5	4
Average for unfertilized plats.	9	4	4	2	3	2	3	2	4	5	15	38	67	194	181	49	51	16	4	3

* c = Unfertilized plats.

of the table is the average for all plats on the dates analyzed, and below that is the average for all unfertilized and unmanured plats. The last, by eliminating the effect of nitrate fertilizers, is a better index to the normal course of nitrification than is the average of all the plats.

The timothy had been on the land for two years previous to 1907 and there was a good stand. Commercial fertilizers had been added to each of the previous crops, and farm manure once. For the crop of 1907, farm manure was applied as a top-dressing on October 2, 1906, to the plats designated in Table 1, and commercial fertilizers were applied on April 23 and 24, 1907. Soil samples were taken on April 23, immediately before the fertilizers were applied. The timothy was cut on July 23 and samples for nitrates were taken the next day. The maize was planted on May 29 and cut on September 24. No fertilizer nor manure was applied to either the maize or the oats. The oats were drilled in on April 23, the day following the first sampling for nitrates. They were harvested on August 6 and samples were taken on the following day.

Seasonal range of nitrates under timothy, maize, and oats

A striking feature of the nitrate content of the timothy plats is the uniformity and scarcity of nitrates throughout the growing season. In all except the nitrate-fertilized plats the nitrate content was higher in April and August than during the period of more active growth. The land had not been plowed for two years and was covered with a thick sod, neither of which conditions was favorable for nitrification. The seasonal range of nitrates under timothy resembled that under alfalfa in the experiments of King and Whitson and in those of Stewart and Greaves. During the first half of May, when growth first became active, there was a tendency for the nitrates to increase, as is shown by the analyses of May 3 and May 14; there was then a decrease during the remainder of May and during June, with a slight increase at the time of harvest which was continued in the middle of August when the aftermath was growing.

The timothy sod was plowed under in the autumn of 1907, but nitrification had not proceeded very rapidly up to the time of the first analyses on May 19, 1908. The most noticeable increase was between July 6 and July 27, 1908. At the latter date there was a very large accumulation of nitrates in the soil, probably the maximum of the season. This period of active nitrification was also the time during which the maize was making its most active growth. The weather was warm and very favorable for the growth of maize, which was an unusually good crop this year. It is a striking fact that in spite of the pronounced growth of the maize at this time the nitrates increased at a rate which gave no appearance of their being used by the crop. The nitrate content under the crop and on the unplanted soil is shown graphically in Diagram I.

Between July 28 and August 10 there was a perceptible decline in the nitrate content, and between August 10 and September 17 this decline was very marked. It is strange that the diminution in nitrates should take place after that period of growth during which the maize plant has been shown to absorb fully one half of the nitrogen that it utilizes.

The analyses of April 22, 1909, which were made before the oats were planted, show a much higher nitrate content in the soil in the spring following maize than in that following timothy, although the analyses were made nearly a month earlier in 1909 than in 1908. Between April 22 and June 24 there was a considerable loss of nitrates, as is to be expected in view of the fact that the nitrogen absorption of the crop is greatest at that time. The lowest figures for nitrates, however, were on July 12 and August 7, although the crop could not have been making very heavy drafts on the former date and had been harvested before the latter date, and in the face of the fact that nitrification was at its maximum at those dates under the maize crop.

It would seem as if nitrate production is most pronounced during the active growth of the crop on the soil, and decreases with the less vigorous growth of the crop. The characteristic relations of the nitrate content of the soil under timothy, maize, and oats are shown in Diagram II.

Nitrates on the planted and the unplanted sections of the plats

As before stated, a part of each plat was left unplanted in 1908 and 1909, but was otherwise given, as closely as possible, the same treatment as was given to the planted section of the plat. When the planted section of the plat was in maize the unplanted section was given the same cultivation; when oats were growing on the planted section, the unplanted section was kept free from weeds by scraping the surface with a hoe. In 1908 unplanted sections were maintained on the north as well as on the south end of each plat for a distance of twenty-five feet from each end.

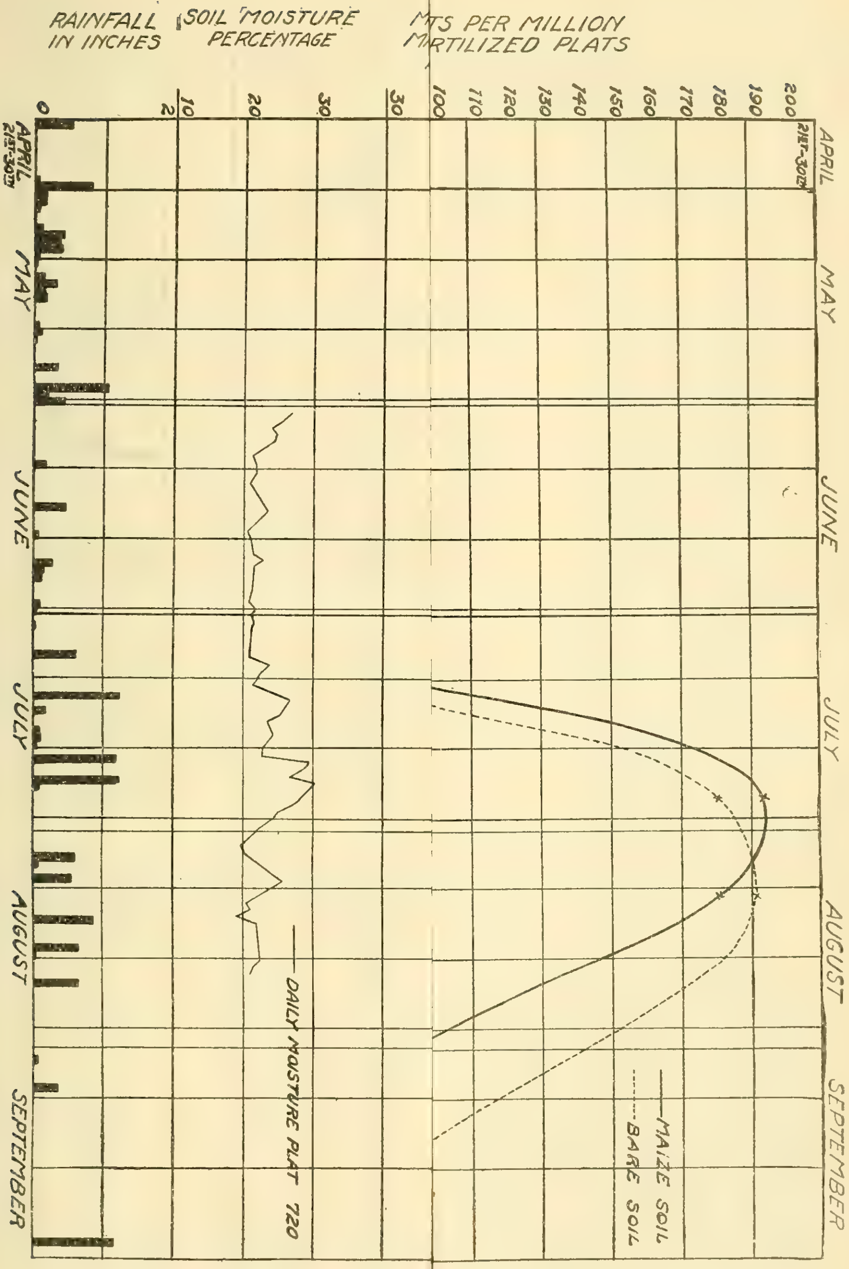


DIAGRAM I.— *Nitrates and moist infall during the growing season*

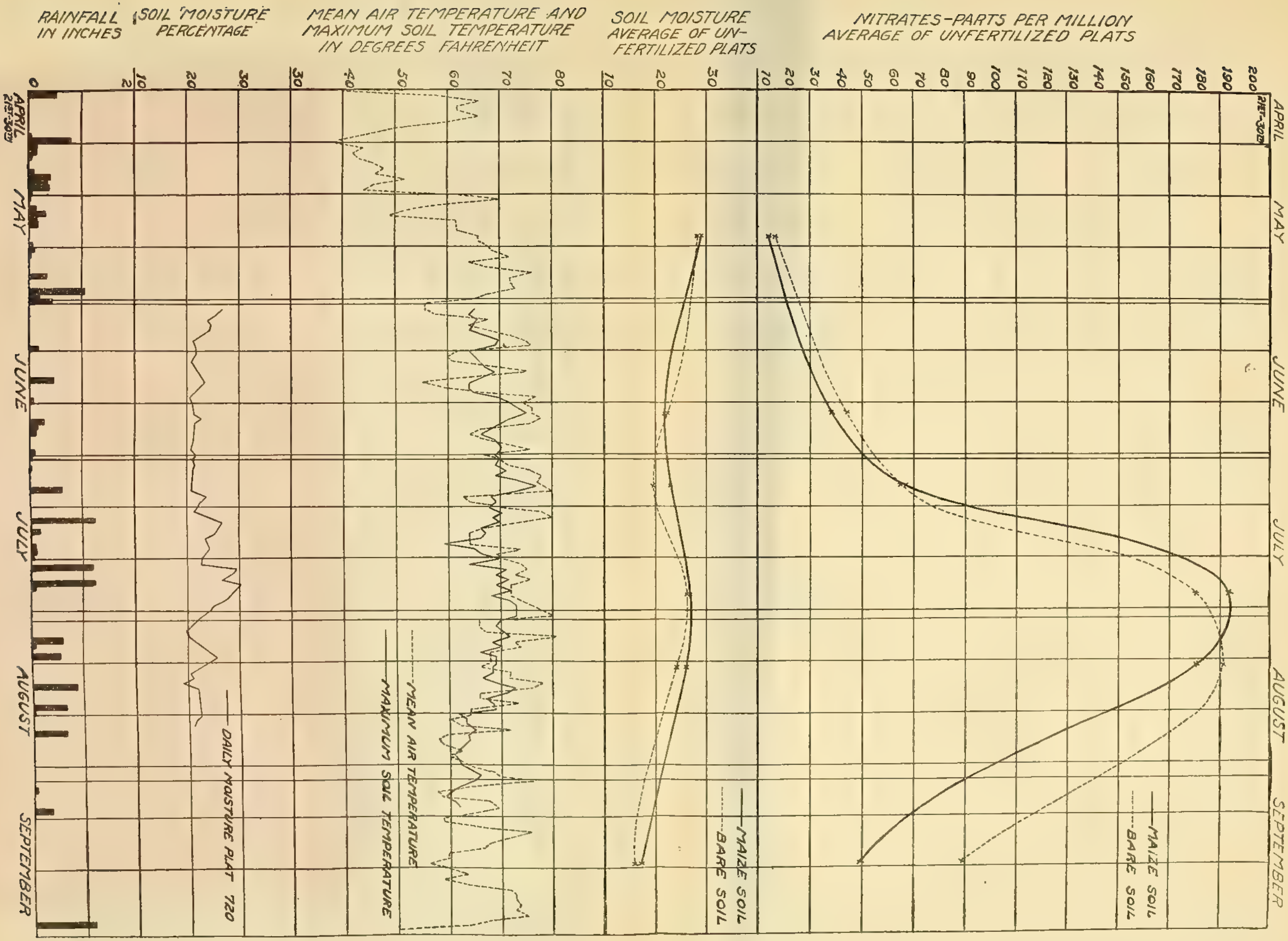


DIAGRAM I.— *Nitrates and moisture in soil planted to maize and in unplanted soil in 1908, and air and soil temperatures and rainfall during the growing season*

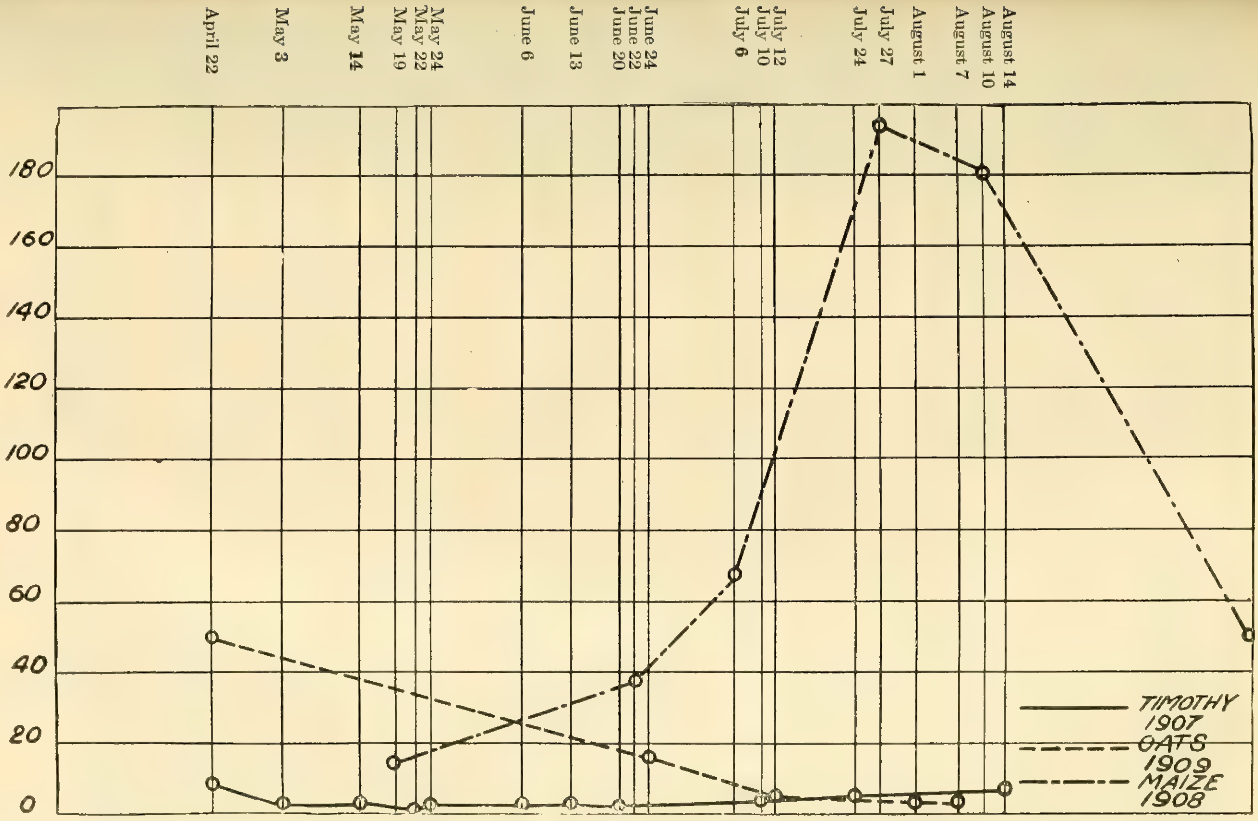


DIAGRAM II.— *Nitrates under timothy, maize, and oats, on unfertilized plats, during the seasons 1907, 1908, and 1909*

When the unplanted section at the north end was sampled, the north half of the planted area was sampled on the same date; and the same was done with the south part of the plat. The north and south halves were sampled alternately. In 1909 all samples were taken from the south halves of the plats, the unplanted and the planted parts corresponding exactly with those of the previous year. These are referred to as "north ends," "south ends," "north middles," and "south middles."

A comparison of the nitrate content of the unplanted and the planted sections of the same plat serves to indicate what has been the effect of the crop on the nitrate content of the soil. These figures for the years 1908 and 1909 are given in Table 3.

A striking feature of the nitrate content of the soil during the growth of maize is the close agreement between the nitrates in the planted and those in the unplanted sections of the plats. This is particularly noticeable in the analyses of July 27, previous to which the maize crop must have absorbed about one half of its nitrogen, judging by what is known of the life history of the plant. The previous heavy draft on soil nitrates is indicated by the analyses of June 22 and July 6, which show a lower nitrate content in the planted sections of the plats. The analyses of July 27 indicate either that the production of nitrates is proceeding much more rapidly in the cropped than in the uncropped soil, or that the maize crop is absorbing part of its nitrogen in some form other than as nitrates.

The analyses of August 10 show a decline of the nitrates on the planted areas, while on the unfertilized plats the nitrates still continue to increase on the unplanted spaces. From the standpoint of nitrogen utilization by the crop, the decline in nitrates at this late date is difficult to explain.

The analyses of April 22, 1909, made before the oats were planted, show a continuation of the more rapid decline on the maize land than on the uncropped spaces. The next analyses, June 24, show a marked decline in the nitrate content of the cropped sections of the plats, as do also those of July 12. By the latter date the nitrogen utilized by the oat crop has in large part been absorbed. The last analyses, however, on August 7, show a still greater decrease in the nitrate content, when a partial recovery might be expected and when the nitrate content of the uncropped soil has made a very great increase.

Comparing the two crops, the following facts are significant:

(1) When nitrification is at its maximum in the maize land the cropped

TABLE 3. NITRATES IN UNPLANTED SECTIONS (ENDS) AND PLANTED SECTIONS (MIDDLES) OF PLATS 711 TO 732 FOR TWO YEARS (PARTS PER MILLION)

Plat	Maize, 1908										Oats, 1909							
	May 19		June 22		July 6		July 27		August 10		April 22		June 24		July 12		August 7	
	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted
711c*	17	25	33	40	64	62	158	182	179	162	64	94	59	38	36	8	63	10
712	9	10	50	31	74	53	194	172	176	162	86	61	52	14	56	7	76	3
713	35	14	38	40	63	59	198	172	120	201	75	44	77	25	39	9	61	10
714c	15	22	54	52	63	90	221	268	188	238	86	60	35	18	127	7	56	8
715	29	26	44	57	50	60	221	211	164	158	86	56	93	10	129	8	83	11
716	30	33	45	69	42	47	255	261	205	156	114	32	49	5	70	6	76	8
717c	21	20	64	43	46	39	202	211	231	234	112	45	74	16	24	3	86	2
718	14	12	48	36	45	40	147	224	156	141	82	57	41	3	48	2	79	2
719	19	21	40	30	53	30	176	178	195	160	99	66	58	19	38	2	94	3
720c	19	17	43	31	70	102	156	211	185	136	81	50	52	13	35	2	77	3
721	27	20	54	38	98	66	194	130	198	152	77	36	14	3	12	4	119	4
722	25	12	31	32	70	47	194	97	158	139	63	26	70	4	36	2	127	3
723c	20	11	38	47	48	45	147	141	202	198	89	40	65	4	65	4	85	1
724	20	12	47	36	55	50	201	208	185	117	82	30	97	4	41	4	86	2
725	19	21	47	50	54	56	201	194	136	106	80	30	61	3	32	5	100	1
726c	17	11	47	38	40	51	194	228	136	147	50	27	48	5	61	2	66	1
727	17	15	40	49	34	75	174	194	178	172	71	41	25	3	60	3	65	1
728	22	15	56	42	104	81	168	178	154	165	85	59	32	4	68	6	135	2
729c	17	4	35	25	84	101	172	158	188	178	84	45	66	21	60	3	32	1
730c	7	7	43	27	104	50	195	150	221	152	56	44	50	15	46	3	51	1
731	36	14	82	26	47	70	238	198	227	178	106	49	48	9	65	4	67	1
732	45	44	79	64	104	106	304	251	147	185	122	63	58	10	79	6	114	3
Average	22	18	48	41	64	63	196	192	179	165	84	48	56	11	56	5	82	4
Average for unfertilized plats	17	15	45	38	65	67	181	194	191	181	78	51	56	16	57	4	64	3

*c = Unfertilized plats.

soil on the unfertilized plats contains more nitrates than does the uncropped soil.

(2) In oat land the nitrates are never so high in the cropped as in the uncropped soil.

(3) While the maximum nitrate content in the uncropped soil occurred at about the same dates in both years, that in the cropped soil varied with the crops.

(4) Under both crops the nitrate content was higher during the period when the crop was making its greatest draft on the soil nitrogen than in the later stages of growth, and this in spite of the fact that the nitrates in the uncropped soil were increasing while those in the cropped soil were disappearing.

These phenomena are explainable on the assumption that nitrification is stimulated by some processes connected with the active growth and absorbing functions of the plant roots, and is inhibited by the conditions accompanying the decreasing activities of the roots.

Aside from the influence of cultivation and nitrogen absorption, the reason for the large differences in the nitrates under these crops, as well as under the corn, potatoes, alfalfa, and oats grown by King and Whitson and by Stewart and Greaves, may be sought in the inherent differences between plants of different species as regards their stimulating or inhibiting effect on nitrate formation. Another factor that may be operative in determining the quantity of nitrates is the habit of the plant in respect to the form in which it absorbs nitrogen. It is conceivable that a plant may induce an active nitrification in the soil and yet absorb its nitrogen largely in the form of organic nitrogen.

The response of different plants to nitrogen in different forms indicates a difference in their abilities to utilize this material when in certain compounds. For instance, grass makes a quick and effective response to sodium nitrate, and the nitrate content of the soil, unless heavily dressed with nitrate fertilizer, is maintained at a very low point. Oats are less responsive to the nitrate fertilizer and do not reduce the nitrate content of the soil so low. Maize is but little affected by a nitrate fertilizer, while it is greatly benefited by fresh applications of farm manure. The nitrates, meanwhile, are present, apparently greatly in excess of the needs of the plant.

Relation of nitrates to soil moisture on the plats

Moisture determinations were made in all soil samples in which nitrates were determined; in addition, daily determinations of moisture were made in certain of the unfertilized plats during the summers of 1907 and 1908. It was thought that a record of the moisture content for a period preceding the nitrate determinations would show the relation between soil moisture and nitrate formation better than would a statement of the moisture content on the day on which nitrates were determined. The nitrate content and the average moisture content of the soil for the time intervening between the dates on which nitrates were determined are given in Table 4:

TABLE 4. AVERAGES OF DAILY DETERMINATIONS OF SOIL MOISTURE FOR THE PERIODS INTERVENING BETWEEN NITRATE DETERMINATIONS ON CROPPED SOIL

Timothy, 1907

	June 4 to 6		June 7 to 13		June 14 to 20		June 21 to July 24		July 25 to August 14	
Plat	711	720	711	720	711	720	711	720	711	720
Moisture (percentage)	22.8	26.3	23.4	26.3	23.8	27.0	21.7	25.1	17.9	21.2
Nitrates (parts per million)	3	2	5	2	4	1	5	3	7	6

Maize, 1908

	June 2 to 22		June 23 to July 6		July 7 to 27		July 28 to August 10	
Plat	711	720	711	720	711	720	711	720
Moisture (percentage)	22.7	22.6	21.1	21.5	24.0	25.3	22.1	22.8
Nitrates (parts per million)	40	31	62	102	182	211	162	136

In 1907 the moisture content was perceptibly lower and the nitrates were higher in plat 711 than in plat 720. In 1908 there was little difference in moisture in the two plats and no consistent difference in nitrates. In the latter part of July and the first part of August there was a decrease in moisture content in both plats each year; in 1907 this was accompanied by an increase in nitrates, while in 1908 a decrease in nitrates occurred. The drying out of the soil and consequent aeration does not appear to produce the same result in both cases, from which it may be taken for granted that factors other than this are more important in nitrate formation. These figures are shown graphically in Diagram I.

Changes in soil moisture.— While the gradual changes in soil moisture content that have been examined do not appear to have constituted an important factor in promoting nitrate formation — at least not sufficient to prevail over other influencing conditions — the question arises whether the more frequent, although less marked, changes in moisture content due to the effect of transpiration from the plant in drying out the soil between rains would not cause greater nitrification on the planted than on the unplanted sections of the plats.

Analyses made in the course of the experiments here described give the daily moisture content of the planted sections on certain plats throughout the growing season. There were also moisture determinations made in each of the soil samples in which nitrates were determined, and these show to what extent the cropped soils dried out as compared with the uncropped soils. These data are given in tables 5 to 7:

TABLE 5. Daily Determinations of Moisture in Soil under Timothy, 1907
(Percentage of Dry Soil)

Date	Plat 711	Plat 720	Plat 728	Plat 729
June				
6	23.3	27.3	25.0	27.5
7	30.7	32.6	29.9	34.5
8	25.0	27.5	24.5	28.0
11	21.2	23.4	20.7	23.4
12	20.9	23.7	....	23.4
13	19.3	24.5	20.6	23.3
14	19.0	23.0	18.9	21.7
15	18.9	23.4	17.6	22.8
17	23.0	24.6	20.6	25.4
18	14.9	18.6	16.6	18.9
19	30.3	32.6	31.0	32.4
20	37.2	40.0	37.1	41.8
21	35.1	38.8	36.8	40.2
22	21.0	24.0	22.2	25.6
24	18.7	22.1	19.3	22.8
25	18.6	22.4	17.3	23.6
26	22.4	24.6	21.9	27.3
27	22.8	20.3	18.6	23.8
28	20.6	....	19.6	25.3
29	19.4	21.7	17.7	22.2

TABLE 5 (*continued*)

Date	Plat 711	Plat 720	Plat 728	Plat 729
July				
1	26.4	29.7	28.2	31.0
2	27.5	31.0	29.3	29.5
3	25.6	28.2	27.5	27.5
5	24.2	27.0	25.3	27.0
6	22.5	25.4	24.0	25.7
8	20.0	23.7	23.3	23.8
9	19.6	23.6	21.6	23.7
10	18.7	20.7	19.3	21.5
11	19.4	20.6	20.9	19.7
12	25.9	31.2	29.9	30.2
13	26.4	29.2	28.2	29.2
15	23.3	26.1	23.7	26.6
16	20.9	23.4	23.3	24.0
17	20.0	24.2	23.7	25.1
18	21.9	25.1	23.7	26.4
19	18.9	22.1	19.7	22.1
20	18.7	22.6	20.3	22.1
22	19.0	20.9	20.4	24.2
23	15.6	20.9	19.0	22.5
24	16.0	20.1	18.7	21.2
26	16.8	20.0	19.6	21.7
27	16.6	19.3	16.0	20.7
29	15.6	19.7	17.5	21.3
30	14.9	18.7	16.8	20.0
31	14.5	21.2	17.6	19.6
August				
1	16.1	19.4	17.5	21.2
2	20.1	25.3	23.4	25.6
3	21.0	23.3	23.8	24.6
5	21.3	22.6	23.0	23.6
6	19.7	24.2	24.0	25.4
7	19.4	23.0	22.5	23.1
8	18.6	21.9	22.6	23.6
9	18.7	22.5	22.2	23.1
10	18.9	21.0	20.9	22.5
12	17.5	19.9	20.4	19.1
13	18.3	19.3	21.0	21.2
14	16.6	20.1	18.7	20.1
15	15.7	17.9	17.6	19.9
16	16.2	17.3	17.7	18.9
17	15.2	17.2	16.4	17.5
19	14.8	16.4	13.3	15.0
20	12.4	16.5	15.2	15.3

TABLE 6. DAILY DETERMINATIONS OF MOISTURE IN SOIL UNDER MAIZE, 1908
(PERCENTAGE OF DRY SOIL)

Date	Plat 711	Plat 720	Plat 728	Plat 729
June				
2	27.3	26.8	26.5	26.5
3	25.1	25.7	26.1	24.2
4	26.1	24.0	25.0	24.2
5	24.6	24.8	24.5	23.8
6	23.8	24.5	23.6	23.3
8	21.9	21.2	22.2	20.3
9	20.3	21.6	22.8	21.3
10	20.9	21.7	22.6	21.5
11	22.1	21.5	21.7	20.6
12	20.3	20.9	21.2	21.2
13	23.6	21.5	23.8	20.7
16	22.5	23.4	23.4	22.8
17	21.6	22.4	22.8	22.4
19	20.0	20.7	22.8	22.1
20	22.1	21.0	21.3	20.7
22	21.5	21.2	22.4	20.9
23	21.0	22.6	22.2	21.5
24	21.5	21.7	22.2	21.3
25	20.6	21.5	23.3	21.5
26	20.6	21.3	22.6	21.5
27	21.7	21.2	23.1	21.7
29	20.1	20.9	23.3	20.6
30	21.6	21.7	22.5	21.3
July				
1	21.5	21.2	22.6	20.7
2	20.7	21.5	....	....
3	21.3	21.2	22.4	20.0
7	20.9	21.0	21.5	20.9
8	23.1	23.4	24.6	23.1
9	22.5	22.6	23.8	22.1
11	24.0	21.7	21.7	21.3
13	26.8	26.7	27.5	26.1
15	23.1	25.1	24.2	25.0
16	21.0	23.6	23.7	23.3
18	22.5	24.2	24.2	22.8
20	21.7	22.8	23.8	22.2
21	21.7	22.6	23.7	22.6
22	26.4	29.7	28.7	28.0
23	26.4	28.8	27.8	27.2
24	24.2	26.8	26.5	25.6
25	28.8	30.3	31.9	27.8
27	26.8	28.5	26.7	26.7
28	24.8	27.0	27.2	27.5
29	23.6	25.1	25.1	23.0
30	23.4	24.2	24.2	22.8
31	22.4	22.5	24.3	22.1

TABLE 6 (*continued*)

Date	Plat 711	Plat 720	Plat 728	Plat 729
August				
3	19.3	19.9	21.0	19.6
4	18.7	20.3	21.3	19.1
5	23.0	21.9	23.4	23.0
6	22.1	23.1	24.6	23.1
8	24.5	25.6	25.7	25.9
11	21.0	20.7	23.1	23.0
12	20.9	21.0	23.0	21.3
13	18.2	19.0	21.6	20.9
14	21.3	22.2	23.1	22.2
18	22.5	22.4	24.5	24.2
19	21.2	22.4	24.6	21.7
20	21.6	21.7	23.0	21.7
21	20.0	21.0	21.9	21.7

TABLE 7. AVERAGE MOISTURE CONTENT FOR PLANTED AND BARE SECTIONS OF MAIZE PLATS ON DATES DESIGNATED (PERCENTAGE OF DRY SOIL)

Date	Plat 711		Plat 720		Plat 728		Plat 729	
	Un-planted	Planted	Un-planted	Planted	Un-planted	Planted	Un-planted	Planted
June								
3	27.0	25.1	......	......	......	......	......	......
4	......	......	23.3	24.0	......	......	......	......
5	......	......	......	......	25.0	24.5	......	......
6	......	......	......	......	......	......	22.6	23.3
9	21.6	20.3	......	......	......	......	......	......
10	......	......	21.0	21.7	......	......	......	......
11	......	......	......	......	23.1	21.7	......	......
12	......	......	......	......	......	......	21.5	21.2
16	23.6	22.5	......	......	......	......	......	......
17	......	......	21.9	22.4	......	......	......	......
19	......	......	......	......	24.5	22.8	......	......
20	......	......	......	......	......	......	21.0	20.7
24	22.5	21.5	......	......	......	......	......	......
25	......	......	21.5	21.5	......	......	......	......
26	......	......	......	......	23.1	22.6	......	......
27	......	......	......	......	......	......	22.1	21.7
30	21.7	21.6	......	......	......	......	......	......
July								
1	......	......	20.6	21.2	......	......	......	......
2	......	......	......	......	23.3	......	......	......
3	......	......	......	......	......	......	21.3	20.0
8	24.6	23.1	......	......	......	......	......	......

TABLE 7 (*continued*)

Date	Plat 711		Plat 720		Plat 728		Plat 729	
	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted
July (*continued*)								
9	......	......	23.4	22.6	......	......	......	......
11	......	......	......	......	23.4	21.7	......	......
13	......	......	......	......	......	......	28.2	26.1
16	24.5	21.0	......	......	......	......	......	......
18	......	......	23.4	24.2	......	......	......	......
20	......	......	......	......	25.0	23.8	......	......
21	......	......	......	......	......	......	23.0	22.6
23	28.0	26.4	......	......	......	......	......	......
24	......	......	26.4	26.8	......	......	......	......
25	......	......	......	......	31.7	31.9	......	......
27	......	......	......	......	......	......	27.2	26.7
29	24.5	23.6	......	......	......	......	......	......
30	......	......	22.6	24.2	......	......	......	......
31	......	......	......	......	24.6	24.3	......	......
August								
3	......	......	......	......	......	......	20.0	19.6
5	21.0	23.0	......	......	......	......	......	......
6	......	......	21.9	23.1	......	......	......	......
8	......	......	26.7	25.6	......	......	......	......
11	......	......	......	......	23.8	23.1	......	......
12	......	......	......	......	......	......	21.7	21.3
14	23.4	21.3	......	......	......	......	......	......
18	......	......	23.3	22.4	......	......	......	......
19	......	......	......	......	24.0	24.6	......	......
20	......	......	......	......	......	......	22.1	21.7

It is seen from these tables that, while there is considerable range in the maximum and minimum moisture contents of these plats, the changes are not rapid; at least, the drying-out process is slow, and it is the drying that causes aeration in soil. Furthermore, it is quite apparent that on the maize soil, in which nitrification is most active, the changes in the moisture content of the cropped soil do not differ greatly from those on the uncropped soil on the same dates. It is also very evident that the changes in the moisture content continue throughout the summer, and should therefore induce nitrate formation during the ripening stage of the crop growth as well as in the early stages; while Table 2 shows that nitrate production is greater on the planted soil only during the earlier stages of growth.

In diagrams I (page 20), V (page 42), VI and VII (page 52), XVI (page 64), XXVI (page 94), and XXVII (page 95) the moisture and nitrate contents of the soils for the years 1908, 1910, 1911, and 1912 are shown graphically. In these diagrams both moisture and nitrates in the planted and the unplanted sections of the plats are shown for one to three crops each year. The diagrams serve to bring out plainly any related changes that may occur in these two constituents of the soil. These diagrams sometimes show an increase of nitrates accompanying or following a rise in moisture content, and sometimes a decrease, in both the planted and the unplanted soil. A decrease in nitrates may be due to leaching after a very heavy rainfall. This is strikingly shown in the analyses following the heavy rain of September 1, 1912. In most of the years included in these records there was a dry period during the latter part of June or in July, and at this time the soil moisture usually reached its minimum. As the soil moisture increased in August there was generally an increase in nitrates in the unplanted soil; in the planted soil, however, there was much less regularity in this relation and it varied with the kind of plant grown on the soil. The nitrates in oat soil, for instance, were usually not influenced by an increase in moisture content, while under maize they sometimes responded to such an increase.

Some relations between soil temperature and nitrate formation

Records were kept of the soil temperatures taken daily at 4.30 p. m. during the growing seasons of 1907 and 1908, at a depth of six inches on certain unfertilized plats. In Table 8 are given the average temperatures for the periods between the dates on which nitrates were determined, also the nitrate content at the end of the period:

TABLE 8. AVERAGES OF DAILY SOIL TEMPERATURES AT DEPTH OF SIX INCHES FOR THE PERIODS INTERVENING BETWEEN NITRATE DETERMINATIONS ON CROPPED SOIL

Timothy, 1907

	June 7 to 13		June 14 to 20		June 21 to July 24	
Plat	711	720	711	720	711	720
Temperature (Fahrenheit)	60.0°	60.5°	65.3°	66.5°	68.1°	69.2°
Nitrates (parts per million)	5	2	4	1	5	3

TABLE 8 (*continued*)

Maize, 1908	June 2 to 22		June 23 to July 6		July 7 to 27		July 28 to August 10	
Plat	711	720	711	720	711	720	711	720
Temperature (Fahrenheit)	66.8°	65.9°	69.2°	69.8°	69.4°	69.8°	72.2°	71.1°
Nitrates (parts per million)	40	31	62	102	182	211	162	136

In the year 1907 the nitrates were fairly constant in amount throughout the season in these plats, in spite of changes in soil temperature. This is probably because of the heavy draft of the timothy crop on the soil nitrates. In 1908 the nitrates increase in quantity up to July 27, although the average temperature from July 6 to that date is practically constant. From July 28 to August 10 there is a marked rise in temperature in both plats, but it is accompanied by a decrease in nitrates. The temperatures reached in these plats are not high enough to have any depressing effect on nitrification, as has been demonstrated by many experiments.

In diagrams I (page 20), V (page 42), VI (page 52), and XVI (page 64) are shown the daily mean air temperatures, and in some seasons the daily soil temperatures, and the nitrate contents of the soil for the years 1908, 1910, 1911, and 1912. In the unplanted soil the nitrates usually increased in the spring with the rise in temperature. This continued until July, after which there was apparently little relation between temperature and nitrate content until late autumn. For instance, in 1911 and 1912 there was a decrease in the temperatures from the first half of July to the end of August, while the nitrate content of the unplanted soil increased steadily during that period. The planted soil cannot be said to show any consistent relation between temperature and nitrate content during midsummer.

Experiments with maize in which other plants were sown

In 1907 and 1908 certain plats were planted to maize, between the rows of which the seeds of other plants were sown broadcast. A part of each plat was not planted to maize, but when the other seed was sown between

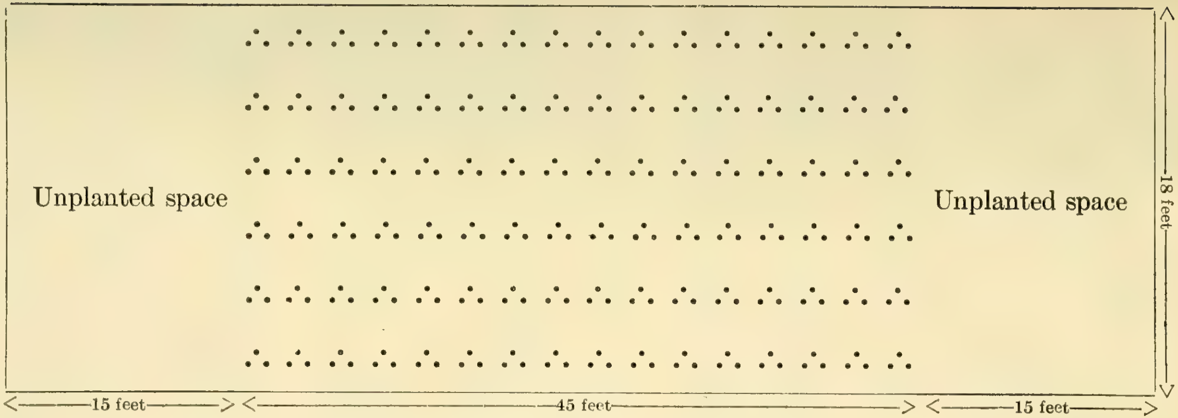


DIAGRAM III.—*Arrangement of maize plats in 1907*

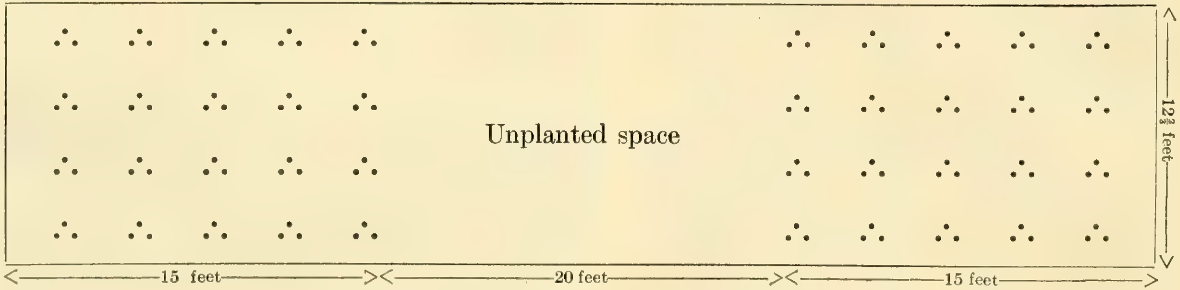


DIAGRAM IV.—*Arrangement of maize plats in 1908* the maize rows it was sown also on the part of the plat on which no maize was planted. In 1907 maize was in the center section of the plat and the sections not planted to maize were on the two ends. In 1908 maize was on the end sections and the soil in the middle was left unplanted. These plats were not on the same soil in 1908 as in 1907. In 1907 the plats were on a sandy soil, while in 1908 they were on the clay loam on which the experiments already discussed were conducted. The arrangements of these plats are shown in diagrams III and IV.

The soil samples were taken with a soil auger to the depth of seven inches. Each sample in 1907 was obtained in the following manner: six borings were taken, carefully mixed on a tablet in the field, placed in a tightly closed jar, and carried to the laboratory for moisture and nitrate determinations. In 1908 four borings were made in each end of the plat, the eight borings from the two ends of the same plat were mixed, and a part was taken as a sample. Samples from the middles were composed of six borings.

Experiments with maize, millet, and soy beans in 1907

Maize was planted in hills three feet apart each way. Five kernels were planted in each hill and these were later thinned to three stalks in a hill. Millet was sown on the ends of certain plats and soy beans on the ends of other plats. These were sown when the maize was cultivated on the dates stated in Table 10. Every third plat was a check plat and was planted only to maize, the weeds in these check plats being kept down by cultivation. The unplanted ends received the same cultivation as the remainder of the plat. Nitrates on the planted and the unplanted sections are given in Table 9.

The analyses were all made late in the growing season, when the growth of maize had reduced the nitrate content in the planted sections below that in the bare sections. The nitrate content of the unplanted soil is fairly constant until late in the season, when, conditions in September evidently being favorable for the process of nitrification, the average for September 16 shows that the nitrates in the unplanted soil have more than doubled since the analyses of August 25. This may have been due to the abundant rainfall during the early part of September, there having been 2.53 inches between September 1 and September 16, while

TABLE 9. NITRATES IN MAIZE AND IN UNPLANTED SECTIONS OF PLATS 1, 4, 7, 10, 13, IN 1907 (PARTS PER MILLION)

Plat	August 5		August 19		August 25		September 16	
	Maize	Un-planted	Maize	Un-planted	Maize	Un-planted	Maize	Un-planted
1	52	90	19	72	15	70	13	130
4	49	111	16	84	16	92	15	208
7	63	96	9	54	10	80	36	143
10	24	73	14	50	31	69	31	165
13	18	56	30	79	41	69	55	183
Average	41	85	18	68	23	76	30	166

there was only .73 inch between August 1 and August 31. On September 9 there was a rainfall of 1.06 inch; however, although this soil is very sandy, there could have been little leaching of nitrates judging from the nitrate content on September 16.

Notwithstanding the very favorable conditions for nitrification and the large nitrate content of the unplanted soil, there is practically no increase of nitrates in the planted soil. This is a striking exemplification of the hypothesis previously advanced, namely, that at least certain plants in some way inhibit the formation of nitrates in the soil during the later stages of plant growth. It is certainly impossible to account for the failure of the nitrates on the cropped soil to increase by supposing that they were leached out of the topsoil, as they would not have remained practically constant while the nitrates in the uncropped soil doubled.

The plats sown to millet and to soy beans afford an opportunity to study the effect of these crops on the nitrate content of the soil by comparing the ends of the plats which, as already explained, were sown to either millet or soy beans alone, with the unplanted ends of contiguous plats. Such a comparison is made in Table 10.

There seems to be little tendency for the nitrates to increase under either of these crops during the early stages of growth; yet those crops that were planted after the first analysis was made, thus permitting frequent observation, display something of this tendency. Thus, on

TABLE 10. NITRATES IN MILLET OR SOY BEANS ALONE AND IN UNPLANTED SECTIONS OF ADJACENT PLATS IN 1907 (PARTS PER MILLION)

Date	Plat 5 Millet sown June 18	Plat 4 Un-planted	Plat 6 Millet sown July 24	Plat 7 Un-planted	Plat 8 Millet sown August 6	Plat 10 Un-planted	Plat 9 Soy beans sown July 5	Plat 11 Soy beans sown July 24	Plat 13 Un-planted
July 9	85	98	79	99	100	54	99	53	57
July 15	73	88	78	82	62	34	60	26	30
July 22	30	110	114	78	101	84	76	74	82
July 29	7	92	73	68	80	52	19	54	60
August 5	2	111	135	96	135	73	9	57	56
August 12	6	96	82	84	111	62	4	75	79
August 19	1	84	60	54	84	50	3	55	79
August 25	2	92	41	80	94	69	2	55	69
September 2	1	96	25	74	66	88	2	52	90
September 16	2	208	6	143	45	165	10	145	183
	Yield per acre		Yield per acre		Yield per acre		Yield per acre	Yield per acre	
	4,999 pounds dry matter		2,026 pounds dry matter		772 pounds dry matter		3,700 pounds dry matter	894 pounds dry matter	

comparing the nitrates under millet sown on July 24 on plat 6 with those in the check plats 4 and 7, it is seen that the first determination of nitrates after the millet was up showed an increase in all three plats; but the increase under the millet was 62 parts per million, while that under the checks was 19 and 28 parts per million, respectively. The millet sown on August 6 did not make much growth. The decrease in nitrates between the analyses of August 12 and August 19 under the millet on plat 8 was 27 parts per million as against 30 and 12 parts per million on plats 7 and 10, respectively. Plat 9, on which soy beans were sown on July 5, shows about the same nitrate content at each analysis up to July 22 as does its check, plat 7; and plat 11, which was sown to soy beans on July 24, continues about the same as its check until August 19. Neither crop can be said to have been associated with marked increase in nitrates at any stage of its growth, although the nitrate content of the soil under them does not show, in early growth, the decrease that would be expected.

There is the same tendency for nitrates to continue at a low figure throughout the later part of the season under millet, as has already been noted under maize and oats. Although plat 4 shows an increase in nitrates from 96 parts per million on September 2 to 208 parts per million on September 16, plat 5 shows practically no gain, notwithstanding the fact that the absorption of nitrogen by the millet crop had practically ceased at that time. The millet sowings of July 24 and August 6 show the same phenomenon.

Soy beans do not exhibit this property to the same degree. Between the dates mentioned there was a slight increase in nitrates under the sowing of July 5, and a marked increase under the sowing of July 24.

Experiments with maize, millet, and weeds in 1908

The plan of experiments in 1908 was primarily the same as that in 1907; soy beans were not used, however, and there was a difference in the arrangement of the planted and the unplanted sections of the plats and in the character of the soil, all of which have before been noted. Analyses were made only on July 22 and August 31. In Table 11 is given a comparison of the amount of nitrates in the planted and the unplanted sections of the check plats, all of which received the same treatment:

TABLE 11. NITRATES IN MAIZE AND IN UNPLANTED SECTIONS ON PLATS 1, 4, 7, 10, 19, 22, 25, IN 1908 (PARTS PER MILLION)

Plat	July 22		August 31	
	Maize	Unplanted	Maize	Unplanted
1	85	66	99	79
4	66	60	90	82
7	78	64	107	79
10	75	69	105	48
19	172	102	112	82
22	140	143	99	75
25	140	101	126	75
Average	108	86	105	74

The higher nitrate content of the planted soil on July 22 is in line with previous results; a similar relation on August 31 is not, however, as the nitrate content under maize is usually low at that time. The growth of maize was very poor on these plats, the plants growing only three or four feet high and producing only about one third of a crop of fodder. There was consequently a very small absorption of nitrogen as compared with a normal crop.

Millet was sown in the maize and in the middles of certain plats, as in 1907, but was not sown on different dates. A miscellaneous assortment of weed seeds was sown on the ends and on the middles of other plats. In Table 12 is shown the nitrate content of the soil under maize and millet mixed, under maize and weeds mixed, under millet alone, and under weeds alone:

TABLE 12. NITRATES IN SECTIONS OF PLATS IN MAIZE AND MILLET, IN MAIZE AND WEEDS, IN MILLET ALONE, AND IN WEEDS ALONE, 1908 (PARTS PER MILLION)

Plat	July 22		August 31	
	Maize and millet	Millet	Maize and millet	Millet
6	77	44	10	4
21	139	96	45	6
	Maize and weeds	Weeds	Maize and weeds	Weeds
5	84	56	15	8

The yields of maize were extremely poor on these plats. This is an interesting condition, as it gives in some cases about the same yield of maize and millet combined as of millet alone, and the same yield of maize and weeds combined as of weeds alone. This is brought out in Table 13:

TABLE 13. YIELDS ON SECTIONS OF PLATS PLANTED TO MAIZE AND MILLET, TO MAIZE AND WEEDS, TO MILLET ALONE, AND TO WEEDS ALONE (IN POUNDS)

Plat	Crop on section	Yield of maize	Yield of other crop	Total yield on section	Yield on sections calculated to same area
6	Maize and millet	4	50	54	36
	Millet	....	42	42	42
21	Maize and millet	3	44	47	31
	Millet	....	34	34	34
5	Maize and weeds	6	23	29	19
	Weeds	....	18	18	18

The nitrates were uniformly higher in the soil planted to the mixture than in the soil planted to a single crop, in spite of the fact that the draft on the available nitrogen must have been nearly the same on both sections. If it is true that the early stages of plant growth favor nitrification in the soil, and that, as indicated by results already presented, maize is much more effective in this respect than is millet, the higher nitrate content under the mixture of maize and millet than under millet alone is readily accounted for.

This experiment, moreover, eliminates the factor of cultivation, to which the higher nitrate content under maize has usually been ascribed. With the maize plats receiving no more cultivation than the millet plats and producing practically the same total quantity of dry matter, the higher nitrate content of the maize soil must be due to some factor other than cultivation. This again suggests the alternative of a beneficial influence on nitrate production, or the utilization by the maize plant of nitrogen in some other form than that of nitrates.

The excessive supply of nitrates in soil planted to maize

Another significant fact brought out by a study of the nitrates under maize is the great excess of nitrates over the apparent needs of the plant during the first half of the growing season. Under oats and millet there appears to be an increase of nitrates during the very early stages of growth, but this quickly disappears as the growth of the plant proceeds. Under grass there is little or no accumulation of nitrates at any stage during the growth of the first crop of the year. Under maize, however, the nitrates accumulate in apparently excessive quantity up to the middle of the growing season, although during this time the crop is growing actively and absorbing large quantities of nitrogen.

In order to obtain some knowledge of the nitrogen absorption during that period of the growth of the maize plant when nitrates were accumulating in the soil, as compared with its later growth, the figures obtained by Roberts and Wing (1888 and 1890) with the same variety of maize (Pride of the North), and in the same field as that in which the experiments with maize and millet were conducted in 1907, may be quoted. In their experiment of 1888 the maize was planted on May 7 and was out of danger of injury from frosts by September 10. Parts of the crop were cut on different dates, weighed, and analyzed. From the weights and the protein nitrogen content of the crop on certain dates, the amounts of nitrogen removed by the plants on these dates may be calculated; and on the basis of weights and analysis at final harvest, the proportion of the total nitrogen acquired by the plant may be deduced for any of the dates on which the cuttings were made. Estimated in this way the maize plants had absorbed 43 per cent of their nitrogen on July 24 and 63 per cent on August 8. In 1889 the maize was planted on May 12 and was mature by September 24. It had acquired 53 per cent of its total nitrogen on August 2, 50 per cent on August 17, and 67 per cent on August 31. The figures for 1889 are somewhat contradictory, as the proportion of nitrogen taken up on August 17 was less than that on August 2. The figures, as a whole, indicate that at least 50 per cent of the nitrogen utilized by Pride of the North maize in this locality in an average year is absorbed by the end of July. Referring to Table 3, it will be seen that on July 27 the nitrate content of the soil under maize on the unfertilized plats was 194 parts per million, while that on the cultivated soil on which no plants grew was 181 parts per million. Even as late as August 10 the nitrate content under the maize on these plats was 181 parts per million, and on the unplanted soil it was 191 parts per million. It is to be expected that there would be a large accumulation of nitrates in the cultivated and uncropped soil, but why this should be true of the soil carrying a heavy crop of maize, practically one half of the nitrogen of which had been absorbed, it is difficult to understand. The formation of nitrates during this period must have proceeded with tremendous activity if nitrates were the source of nitrogen supply for the crop.

Some conditions indicate that maize, at least during part of its period of growth, utilizes nitrogen rather largely in forms other than as nitrates. Ammonification apparently never proceeds more rapidly than nitrification in this soil, which would bar that form of nitrogen as a very considerable source of supply. Organic nitrogen in some of its soluble forms is the only remaining alternative. The great benefit derived by maize from the application of farm manure suggests the possible use of nitrogen in this form.

During the later growth of the maize plant there is a large falling-off in the nitrate content of the soil under the plants as compared with that in the unplanted soil. This indicates either that nitrates are absorbed by maize in large quantities during the period of growth in which it is obtaining the second half of its nitrogen supply, or that nitrate formation is depressed at that time.

Nitrates in soil planted to maize, potatoes, and oats

Experiments in 1910

During the growing season of 1910, determinations of nitrates were made under maize, potatoes, and oats. These were from four plats planted to each crop, of which two plats were limed and two were unlimed. An unplanted strip ran through the center of each plat. Soil samples were taken on the planted and on the unplanted sections of each plat. The sections are designated as "north ends," "middles," and "south ends." The north ends and the south ends were both planted; the middles grew no crop, but were given the same soil treatment as the remainder of the plat. This consisted in cultivation on the maize and potato plats and in scraping the surface on the oat plats. Soil samples were taken to a depth of four feet on the north ends and on the middles, but to a depth of only one foot on the south ends, except on May 12, when composite samples from the north ends and the south ends were analyzed.

The soil on which these plats were located was a heavy clay loam in the same field as that in which most of these experiments have been conducted. This particular piece of land was not uniform as regards either production of crops or nitrates. Fortunately each crop was grown on four separate plats, and in spite of irregularities in single plats the average of the four plats in each crop gives fairly concordant results.

The weather during the growing season of 1910 was drier than normal and the early part of the season was unusually cool. The yield of oats and of potatoes was about the average, but the maize crop was very poor. The small maize crop was due to the soil rather than to the weather, as no manure had been applied and this soil does not produce good yields of maize unless it is well manured. It will be noticed from Table 14 that the nitrates under maize reached over a hundred parts per million in the surface foot of soil; yet, in spite of this large quantity of soluble nitrogen, the crop was very poor. Potatoes and oats made a much better growth and reduced the nitrates considerably lower. This is another example of the slight response of maize to nitrates on this soil.

Planting was done on the following dates: oats (White Russian) on April 28; potatoes (Enterprise) on May 16; maize (Pride of the North) on May 21. The oats were harvested on August 7 and the maize and potatoes on October 20.

A statement of the average nitrate content of the surface foot of soil on the cropped and the uncropped sections of the four plats planted to each crop is given in Table 14. For the cropped sections the averages include both the cropped ends of the plats. Moisture contents for the same plats and dates are given also.

TABLE 14. AVERAGE NITRATE CONTENT AND AVERAGE MOISTURE IN THE SURFACE FOOT OF SOIL ON THE FOUR PLATS PLANTED TO EACH CROP, ON DATES ANALYZED

Nitrate content (parts per million)

Plat	Crop	May 12		June 1		July 28		August 17		September 9		October 17	
		Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted
3612, 3613 3622, 3623	Maize	66	57	98	62	168	92	179	167	247	273	244	167
3614, 3615 3624, 3625	Potatoes	95	34	122	58	127	145	139	135	99	130	43	145
3616, 3617 3626, 3627	Oats	97	49	125	88	75	84	82	154	67	170	97	211

Moisture (percentage of dry soil)

Plat	Crop	May 12		June 1		July 28		August 17		September 9		October 17	
		Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted
3612, 3613 3622, 3623	Maize	20.9	20.0	25.6	24.5	16.0	16.7	22.1	23.0	22.1	23.0	16.5	17.4
3614, 3615 3624, 3625	Potatoes	21.1	20.7	25.7	24.2	14.5	16.1	20.3	19.6	20.3	19.5	19.0	17.2
3616, 3617 3626, 3627	Oats	22.9	21.6	25.7	25.2	9.2	14.9	18.0	18.2	18.0	18.2	16.2	16.5

An examination of this table shows, so far as the maize crop is concerned, the characteristically higher nitrate content in the planted than

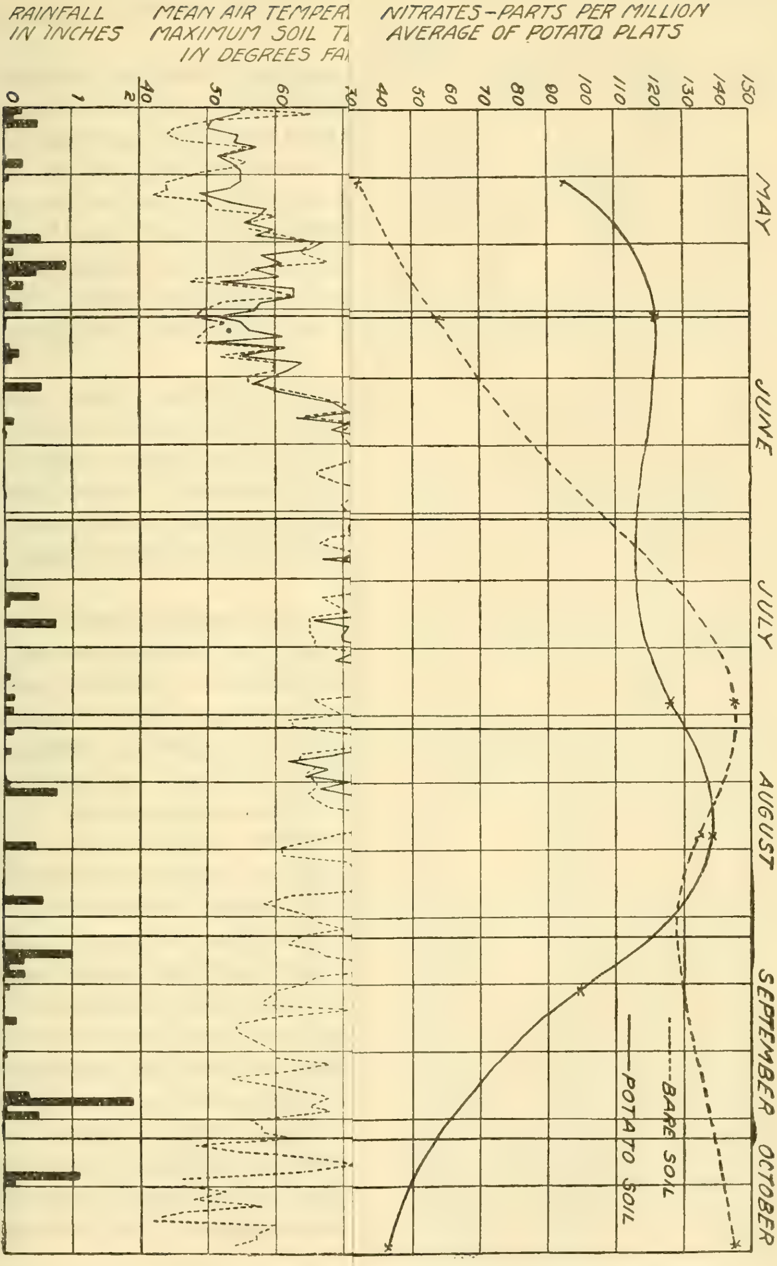


DIAGRAM V.— *Nitrates and moisture and daily rainfall for the growing season*

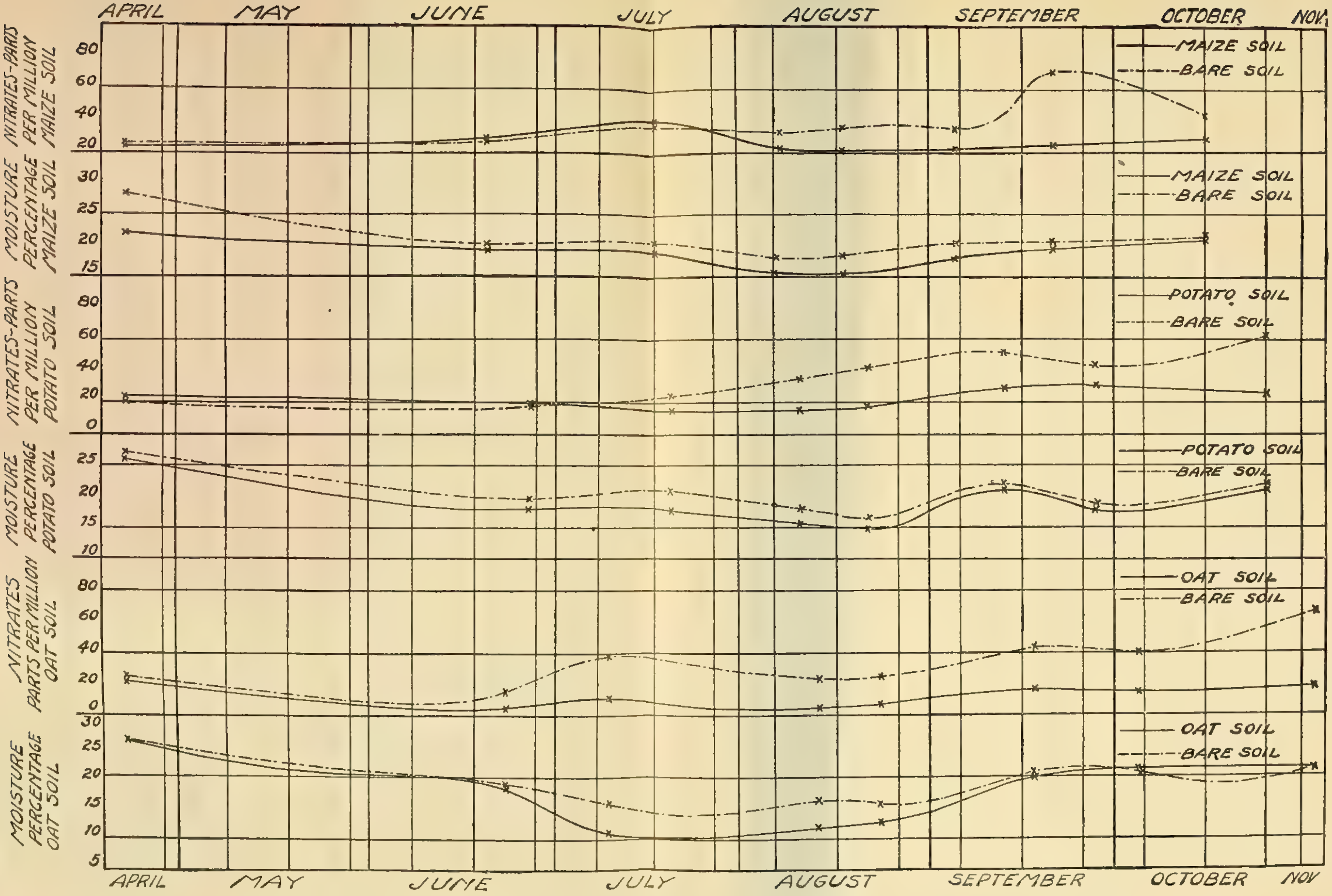


DIAGRAM VII.— *Nitrates and moisture in second eight inches of soil planted to maize, potatoes, and oats and in bare soil, 1911* in the unplanted soil during the latter part of July. The nitrate content of the planted soil was slightly higher than that of the unplanted soil before the crop was seeded, and nitrates continued to develop more rapidly under the maize up to the latter part of July. From that time until the maize was mature in September the nitrates under the crop decreased as compared with those in the unplanted soil, in the usual manner; the analysis of October 17, however, shows a greater relative increase in the planted soil, which is contrary to the hypothesis that has been advanced. It is possible that the very feeble growth of the crop may account for this.

Potatoes, which yielded a normal crop, gave results similar to those obtained from normal yields of maize. The difference in the nitrate content of the potato plats and of the contiguous maize plats on October 17 is very marked. Although the vines were dead at this time, the nitrates were lower under the plants than at any period during their growth, while nitrate formation had again gone forward in the unplanted soil.

Nitrates under the planted and the unplanted sections of the oat plats were of the same relation as in previous years. On the planted sections the nitrates continued to decrease after harvest, as shown by the analysis of September 9 — one month after harvest. A slight increase is shown by the analysis of October 17, but it will be noted that, while nitrates in the planted soil increased 15 parts per million between harvest and October 17, the unplanted soil shows an increase of 57 parts per million in its nitrate content. The course of the nitrates in the planted and the unplanted soil is shown in Diagram V.

Nitrates at different depths.— In order to ascertain what effect the upward and downward movement of nitrates may have on the apparent connection between the plant and the nitrate content of the surface soil, samples were taken on the north ends and on the middles of each plat to a depth of four feet, each foot being analyzed separately. The results of these analyses are shown in Table 15.

The analyses show that nitrates are found to a depth of four feet, but that the changes in nitrate content of the soil are not great beneath the first foot. When changes occur in the second foot, they usually accompany corresponding changes in the surface foot. An increase in the nitrates in the surface foot is seldom accompanied by a decrease in the second

TABLE 15. NITRATES IN EACH FOOT OF THE UPPER FOUR FEET OF SOIL (PARTS PER MILLION)

Crop	Depth (feet)	May 12		July 28		August 17		September 9		October 17	
		Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted
Maize	1	66	57	174	92	201	167	236	273	309	167
Maize	2	34	25	55	47	49	43	79	100	64	57
Maize	3	25	17	41	26	44	34	47	56	43	33
Maize	4	21	15	44	31	42	24	45	72	50	40
Total	....	146	114	314	196	336	268	407	501	466	297
Potatoes	1	95	34	155	145	164	135	110	130	35	145
Potatoes	2	39	19	50	44	63	49	52	60	29	42
Potatoes	3	30	20	37	35	44	38	28	38	25	38
Potatoes	4	31	18	35	31	42	36	28	29	27	37
Total	....	195	91	277	255	313	258	218	257	116	262
Oats	1	97	49	106	84	96	154	84	170	153	211
Oats	2	32	27	37	33	39	48	28	47	51	53
Oats	3	16	12	21	23	28	35	20	46	27	28
Oats	4	31	22	22	23	25	30	22	40	28	33
Total	....	176	110	186	163	188	267	154	303	259	325

foot, as would be the case if the augmented nitrate content of the surface foot was due to an upward movement of the nitrates. On the other hand, the surface and second feet usually increase or decrease in nitrates on the same dates, indicating a downward movement of the nitrates. It would appear from these figures that in the main the nitrate content of the surface foot is determined by the rate of nitrification and by the removal of the nitrates, by various means, from that layer of soil.

The average nitrate content of the maize, potato, and oat plats for the season bears no definite relation to the quantities of nitrogen removed in the crops. In Table 16 the yield of dry matter and of nitrogen in the crops and the average nitrate content of the soil are given.

As usual, the nitrate content is highest under maize, next under potatoes, and lowest under oats. As the nitrogen removed from the soil by the maize crop was less than that absorbed by either of the others, the higher nitrate content of the soil might be considered to be due to that; however, as the potatoes removed more nitrogen than the oats and yet the nitrate content under the potatoes is higher than that under the

TABLE 16. YIELD OF DRY MATTER AND OF NITROGEN IN CROPS, AND AVERAGE NITRATE CONTENT OF SOIL, 1910

Crop	Yield of dry matter (pounds)	Yield of nitrogen (pounds)	Nitrates in first foot of dry soil (parts per million)		Nitrates in planted soil when nitrates in unplanted soil are taken as 100	
			Planted	Unplanted	Planted	Unplanted
Maize	221	3	167	136	123	100
Potatoes	1,910	43	104	108	96	100
Oats	1,670	29	90	126	71	100

oats, the relationship does not hold. Instead, the characteristic relation of the kind of plant to the nitrate content of the soil is again repeated, and this appears to hold, to a large extent, regardless of the relative quantities of nitrogen removed by the crops.

Experiments in 1911

The plat experiments were continued in somewhat the same manner in 1911, but none of the plats were limed. As before, each crop was grown on four separate plats, thus removing to a great extent the error due to local differences in nitrate formation. Another feature introduced in 1911 was the different methods of treatment of maize. Certain plats were mulched with straw, others were scraped with a hoe in order to keep them free from weeds without stirring the soil, and still others received the usual cultivation. The maize was treated also in the following two ways: (1) The crop was harvested on the first of August, the planting having been done at the usual time; the object of the early harvest was to ascertain the effect of the immature plants on the nitrates after the crop was removed. (2) The maize was planted about a month later than on the plats just mentioned and was harvested on August 1. This gave a very immature crop (about eighteen inches high) at harvest

Potatoes were grown as in 1910.

Oats were treated in two ways: (1) on one set of oat plats the land was plowed immediately after harvest and maize was planted; (2) on another set of plats maize was not planted after removing the oats, but the land was plowed and given the same cultivation as was given the maize. The object in giving this treatment after harvesting the oats was to see whether the maize would cause the nitrates to increase in the oat soil and thus overcome the depressing effect of the oats.

The plat treatments may be briefly stated as follows:

Plat	Crop and soil treatment
2201, 2209, 2401, 2409	Maize planted May 20. Mulched
2202, 2210, 2402, 2410	Maize planted May 20. Scraped
2203, 2211, 2403, 2411	Maize planted May 20. Cultivated all summer
2204, 2212, 2404, 2412	Maize harvested August 1. Cultivated all summer
2205, 2213, 2405, 2413	Maize planted June 26, harvested August 1. Cultivated all summer
2206, 2214, 2406, 2414	Potatoes planted May 19. Cultivated all summer
2207, 2215, 2407, 2415	Oats planted May 9. Cultivated after harvest
2208, 2216, 2408, 2416	Oats planted May 9, followed by maize. Cultivated after oat harvest

The temperature during the growing season was nearly normal. There was a deficiency of rainfall, especially during August, at which time the maize suffered from lack of moisture. Oats made a normal growth, as did also potatoes. A killing frost on September 14 stopped growth of all crops on that date.

The soil was manured for all crops in the early spring, at the rate of about five tons of farm manure per acre. This was not sufficient to produce an average yield of maize on the heavy soil of these plats, but the growth of this crop was better than in 1910.

Soil samples for moisture and nitrates were taken to a depth of eight inches from the surface, and also to a depth of eight to sixteen inches. The plats were of the same size as those used in 1910 and each was divided into three sections: the north and the south sections were planted to a certain crop; the middle sections were unplanted and were given the same treatment as the planted sections. Each section of every plat was sampled separately, the sample consisting of three borings.

The results of the determinations of nitrates on the middle sections, representing the unplanted soil, and the averages for the north and south end sections, representing the planted soil, are presented in Table 17. The table gives the nitrates in parts per million for the surface eight inches and for the second eight inches, and the averages of these two depths:

TABLE 17. NITRATES IN SOIL OF PLANTED AND UNPLANTED SECTIONS OF PLATS, 1911 (PARTS PER MILLION)

First eight inches

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209 2401, 2409	Maize, mulched	26	61	59	39	17	5	14	17
2201, 2209 2401, 2409	None	27	54	79	64	46	14	32	18
2202, 2210 2402, 2410	Maize, scraped	32	50	41	121	76	58	71	32
2202, 2210 2402, 2410	None	23	49	53	112	141	135	176	199
2203, 2211 2403, 2411	Maize, cultivated	29	63	105	107	65	45	35	71
2203, 2211 2403, 2411	None	22	60	139	192	174	168	224	210
2204, 2212 2404, 2412	Maize, harvested August 1	30	38	130	116	105	158	139	119
2204, 2212 2404, 2412	None	34	42	140	168	139	215	163	196
2205, 2213 2405, 2413	Maize, planted June 26, harvested August 1	26	34	115	113	100	167	115	209
2205, 2213 2405, 2413	None	31	40	137	109	120	194	160	234
2206, 2214 2406, 2414	Potatoes	33	27	73	39	41	65	67	51
2206, 2214 2406, 2414	None	28	36	127	112	127	211	160	179
2207, 2215 2407, 2415	Oats	23	2	8	8	11	23	26	26
2207, 2215 2407, 2415	None	32	24	42	74	76	123	114	127
2208, 2216 2408, 2416	Oats, followed by maize	26	3	4	6	11	18	15	18
2208, 2216 2408, 2416	None	26	31	56	74	63	168	111	106

Second eight inches

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209 2401, 2409	Maize, mulched	22	29	25	22	15	13	16	13
2201, 2209 2401, 2409	None	19	26	33	29	34	27	50	23
2202, 2210 2402, 2410	Maize, scraped	15	21	28	20	15	12	23	18
2202, 2210 2402, 2410	None	14	21	41	21	27	24	44	42
2203, 2211 2403, 2411	Maize, cultivated	24	28	41	22	21	22	25	30

TABLE 17 (*continued*)

Second eight inches (*continued*)

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2203, 2211 2403, 2411	None	24	26	38	33	35	36	71	45
2204, 2212 2404, 2412	Maize, harvested August 1	29	19	29	27	28	36	42	51
2204, 2212 2404, 2412	None	22	19	39	30	39	42	61	65
2205, 2213 2405, 2413	Maize, planted June 26, harvested August 1	21	15	27	17	28	40	49	49
2205, 2213 2405, 2413	None	23	16	31	19	31	58	70	46
2206, 2214 2406, 2414	Potatoes	26	17	15	16	17	29	31	27
2206, 2214 2406, 2414	None	26	18	25	35	42	53	44	62
2207, 2215 2407, 2415	Oats	20	4	5	5	7	16	14	17
2207, 2215 2407, 2415	None	25	15	38	22	24	43	34	66
2208, 2216 2408, 2416	Oats, followed by maize	19	3	3	3	6	14	10	16
2208, 2216 2408, 2416	None	26	8	21	21	24	52	30	44

Average of first and second eight inches

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209 2401, 2409	Maize, mulched	24	45	42	30	16	9	15	15
2201, 2209 2401, 2409	None	23	40	56	46	40	20	41	20
2202, 2210 2402, 2410	Maize, scraped	23	35	34	70	45	35	47	25
2202, 2210 2402, 2410	None	18	35	47	66	84	79	110	120
2203, 2211 2403, 2411	Maize, cultivated	26	45	73	64	43	33	30	50
2203, 2211 2403, 2411	None	23	43	88	112	104	102	147	127
2204, 2212 2404, 2412	Maize, harvested August 1	29	28	79	71	66	97	90	85
2204, 2212 2404, 2412	None	28	30	89	99	89	128	112	130
2205, 2213 2405, 2413	Maize, planted June 26, harvested August 1	23	24	71	65	64	103	82	129
2205, 2213 2405, 2413	None	27	28	84	64	75	126	115	140

TABLE 17 (*concluded*)

Average of first and second eight inches (*continued*)

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2206, 2214 2406, 2414	Potatoes	29	22	44	27	29	47	49	39
2206, 2214 2406, 2414	None	27	27	76	73	84	132	102	120
2207, 2215 2407, 2415	Oats	21	3	6	6	9	19	20	21
2207, 2215 2407, 2415	None	28	19	40	48	50	83	74	96
2208, 2216 2408, 2416	Oats, followed by maize	22	3	3	4	8	16	12	17
2208, 2216 2408, 2416	None	26	19	38	47	43	110	70	75

A study of the nitrates as recorded in Table 17 shows that the maize, potatoes, and oats bear about the same comparative relation to the nitrate content of the soil that they did in previous years. This characteristic relationship between the crop and the nitrate content of the soil holds also, with some variation due to the season, for the different stages of growth of the plants.

In the first eight inches there is no tendency for the nitrates under the cultivated maize to run higher, at certain periods of growth, than the nitrates in the unplanted soil; but under the maize on the scraped and the late-planted plats the nitrates are higher during a part of the growing season than in the unplanted sections of these plats. In the second eight inches this is also the case.

The effect of all crops, and especially of oats, in depressing the nitrate content during the later stages of growth and afterwards is very marked, even when the nitrates in the unplanted soil continue to increase late into the autumn.

The maize that was harvested on August 1 did not reduce the nitrate content of the soil to the same extent after that date as did the maize not harvested at that time, but there was no tendency for the nitrates under the crop to increase relatively to the nitrates in the bare soil. This may mean that the maize plant uses a considerable quantity of nitrate nitrogen during its later stages of growth, or it may mean that it merely has a depressing effect on nitrification.

Comparing the nitrates in the late-planted and early-harvested plats with those in the early-planted and early-harvested plats, it will be noticed that there is a greater increase in the nitrates in the former after the crop has been removed. Thus the very immature crop does not appear to have as depressing an effect as does the more mature one. A comparison, therefore, of the three sets of plats harvested and planted at different times indicates that it is the later stages of growth that interfere most with the process of nitrate formation.

The nitrates in the planted and the unplanted sections of the plats in cultivated maize, potatoes, and oats for the first and second eight inches of soil are shown in diagrams VI and VII. The moisture content of the soil on all the plats is given in Table 18:

TABLE 18. MOISTURE IN PLANTED AND UNPLANTED SECTIONS OF PLATS, 1911
(PERCENTAGE OF DRY SOIL)

		First eight inches							
Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209 2401, 2409	Maize, mulched	32.1	25.9	22.8	22.0	25.1	22.1	28.0	28.3
2201, 2209 2401, 2409	None	34.1	25.6	23.8	23.4	28.5	22.2	28.8	28.7
2202, 2210 2402, 2410	Maize, scraped	33.4	25.8	16.2	17.9	19.2	23.6	22.5	27.4
2202, 2210 2402, 2410	None	33.6	25.2	16.6	21.4	21.8	25.3	23.8	27.9
2203, 2211 2403, 2411	Maize, cultivated	31.4	23.2	24.9	16.5	17.7	20.0	23.4	26.2
2203, 2211 2403, 2411	None	31.4	24.2	26.6	20.3	20.8	23.6	25.3	26.6
2204, 2212 2404, 2412	Maize, harvested August 1	31.3	22.3	24.5	18.2	18.9	26.7	22.4	26.5
2204, 2212 2404, 2412	None	33.2	23.0	25.6	21.2	21.0	26.9	23.2	26.9
2205, 2213 2405, 2413	Maize, planted June 26, harvested August 1	32.4	21.6	24.0	18.7	19.0	26.4	22.0	27.2
2205, 2213 2405, 2413	None	32.2	22.0	24.7	19.3	19.2	26.3	22.2	28.0
2206, 2214 2406, 2414	Potatoes	32.8	21.6	22.2	15.6	15.9	25.4	21.3	26.1
2206, 2214 2406, 2414	None	34.0	22.7	24.7	19.4	18.7	26.6	22.4	26.8
2207, 2215 2407, 2415	Oats	33.1	18.1	7.2	11.3	12.7	22.3	25.9	24.6

TABLE 18 (*continued*)

First eight inches (*continued*)

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2207, 2215 2407, 2415	None	33.1	19.0	17.2	17.5	16.4	24.3	26.6	25.5
2208, 2216 2408, 2416	Oats, followed by maize	33.0	15.3	7.8	11.7	14.3	24.5	24.2	26.4
2208, 2216 2408, 2416	None	32.7	20.4	16.9	17.8	17.4	25.7	24.9	26.6

Second eight inches

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209 2401, 2409	Maize, mulched	26.6	21.7	19.0	18.3	21.1	19.1	22.6	22.7
2201, 2209 2401, 2409	None	26.6	21.4	19.9	20.9	23.3	18.9	22.9	23.6
2202, 2210 2402, 2410	Maize, scraped	25.4	20.9	16.6	15.9	15.6	19.2	19.0	21.7
2202, 2210 2402, 2410	None	25.6	22.0	17.0	17.9	18.7	21.2	20.2	23.7
2203, 2211 2403, 2411	Maize, cultivated	22.8	19.3	18.9	15.3	15.2	17.8	19.5	21.2
2203, 2211 2403, 2411	None	28.5	20.4	20.8	18.0	18.2	20.5	20.8	21.9
2204, 2212 2404, 2412	Maize, harvested August 1	27.4	18.9	19.2	16.8	17.6	21.6	19.7	21.9
2204, 2212 2404, 2412	None	25.0	18.2	20.2	18.0	18.3	20.8	19.8	21.4
2205, 2213 2405, 2413	Maize, planted June 26, harvested August 1	25.1	18.4	19.4	17.3	17.5	21.2	18.9	21.4
2205, 2213 2405, 2413	None	25.9	18.7	20.7	18.4	18.2	23.2	19.5	21.8
2206, 2214 2406, 2414	Potatoes	25.8	18.0	17.5	15.2	14.9	21.0	18.4	21.4
2206, 2214 2406, 2414	None	26.1	19.6	20.9	18.2	16.8	22.1	18.9	22.3
2207, 2215 2407, 2415	Oats	25.8	18.0	10.9	11.9	12.6	19.6	21.1	21.0
2207, 2215 2407, 2415	None	25.8	18.7	15.7	16.3	15.8	20.5	20.9	21.2
2208, 2216 2408, 2416	Oats, followed by maize	25.7	15.4	11.4	11.8	13.2	20.2	19.3	22.1
2208, 2216 2408, 2416	None	26.0	18.0	16.9	16.9	16.0	21.2	19.7	21.1

TABLE 18 (*concluded*)

Plat	Crop	Average of first and second eight inches							
		April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209 2401, 2409	Maize, mulched	29.3	23.8	20.9	20.1	23.1	20.6	25.3	25.5
2201, 2209 2401, 2409	None	30.3	23.5	21.8	22.1	25.9	20.5	25.8	26.1
2202, 2210 2402, 2410	Maize, scraped	29.4	23.3	16.4	16.9	17.4	21.4	20.7	24.5
2202, 2210 2402, 2410	None	29.6	23.6	16.8	19.6	20.2	23.2	22.0	25.8
2203, 2211 2403, 2411	Maize, cultivated	27.1	21.2	21.9	15.9	16.4	18.9	21.4	23.7
2203, 2211 2403, 2411	None	29.9	22.3	23.7	19.1	19.5	22.0	23.0	24.2
2204, 2212 2404, 2412	Maize, harvested August 1	29.3	20.6	21.8	17.5	18.2	24.1	21.0	24.2
2204, 2212 2404, 2412	None	29.1	20.6	22.9	19.6	19.6	23.8	21.5	24.1
2205, 2213 2405, 2413	Maize, planted June 26, harvested August 1	28.7	20.0	21.7	18.0	18.2	23.8	20.4	24.3
2205, 2213 2405, 2413	None	29.0	20.3	22.7	18.8	18.7	24.7	20.8	24.9
2206, 2214 2406, 2414	Potatoes	29.3	19.8	19.8	15.4	15.4	23.2	19.8	23.7
2206, 2214 2406, 2414	None	30.0	21.1	22.8	18.8	17.7	24.3	20.6	24.5
2207, 2215 2407, 2415	Oats	29.4	18.0	9.0	11.6	12.6	20.9	23.5	22.8
2207, 2215 2407, 2415	None	29.4	18.8	16.4	16.9	16.1	22.4	23.7	23.3
2208, 2216 2408, 2416	Oats, followed by maize	29.3	15.3	9.6	11.7	13.7	22.3	21.7	24.2
2208, 2216 2408, 2416	None	29.3	19.2	16.9	17.3	16.7	23.4	22.3	23.8

There is no material lowering of the moisture content of the maize plats as compared with that of the unplanted soil. In the oat soil the moisture content is markedly lower, as compared with the unplanted soil, in July and August. The nitrate content does not appear to be greatly influenced thereby, as the nitrates during June are slightly lower than in July and August. As has been noted in previous years there appears to be no definite relation in this soil between the nitrate content of the soil under plants and the soil moisture, owing possibly to the fact that the soil was not at any time seriously depleted of moisture.

A comparison of the nitrates in the planted sections of the plats in terms of the nitrates in the unplanted sections, taking the latter in each case as 100, will serve to express more clearly the relation of the different

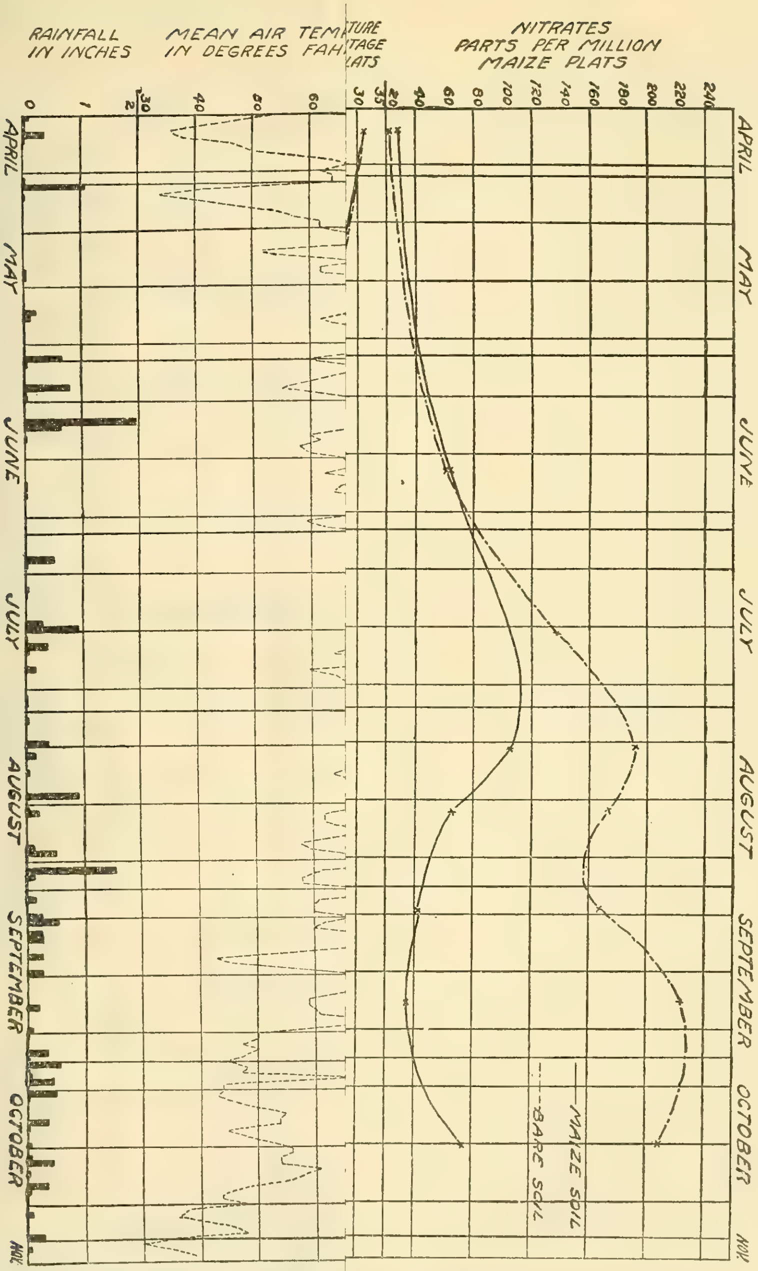


DIAGRAM VI.— *Nitrates and daily air temperature and daily rainfall*

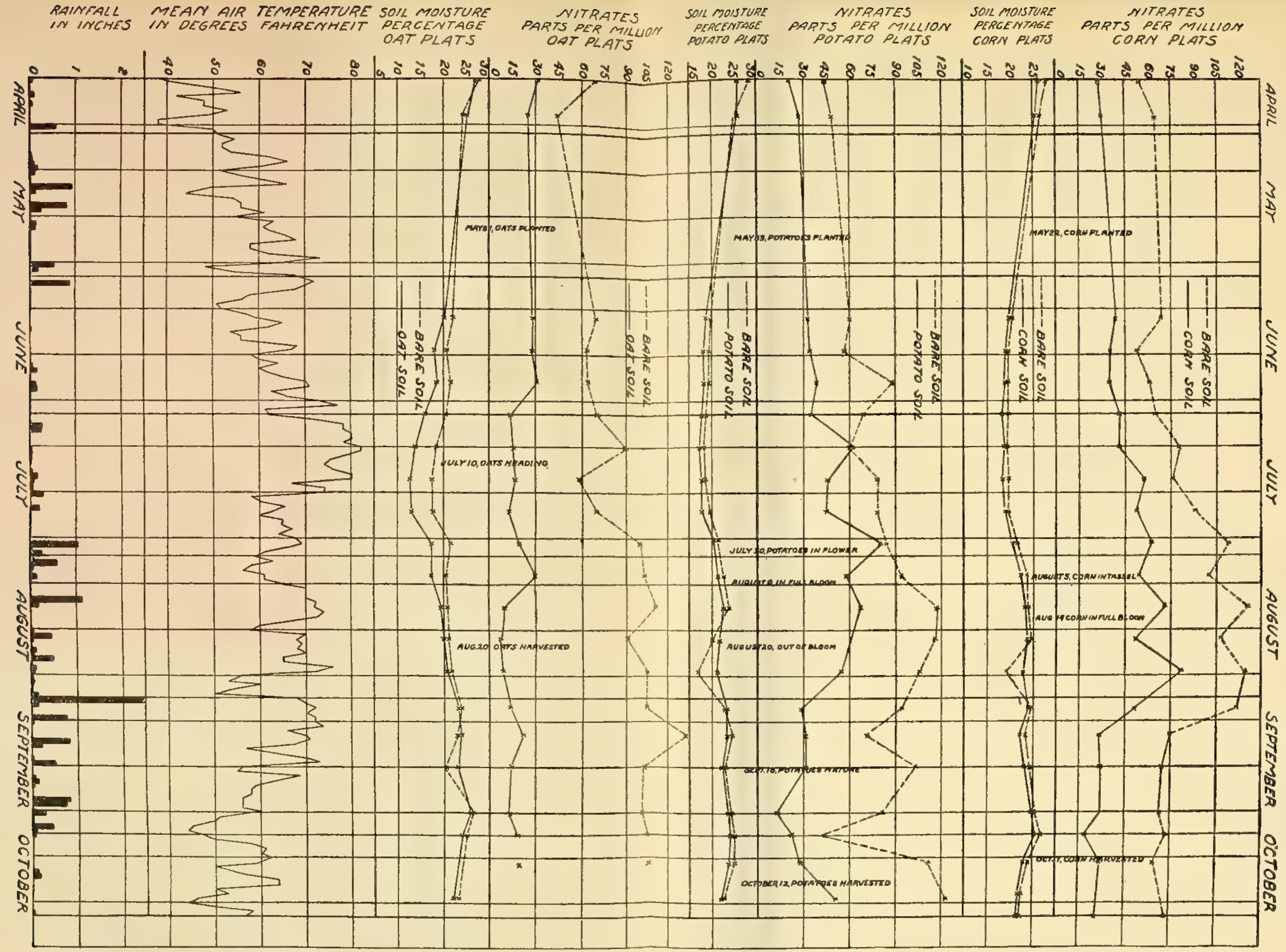
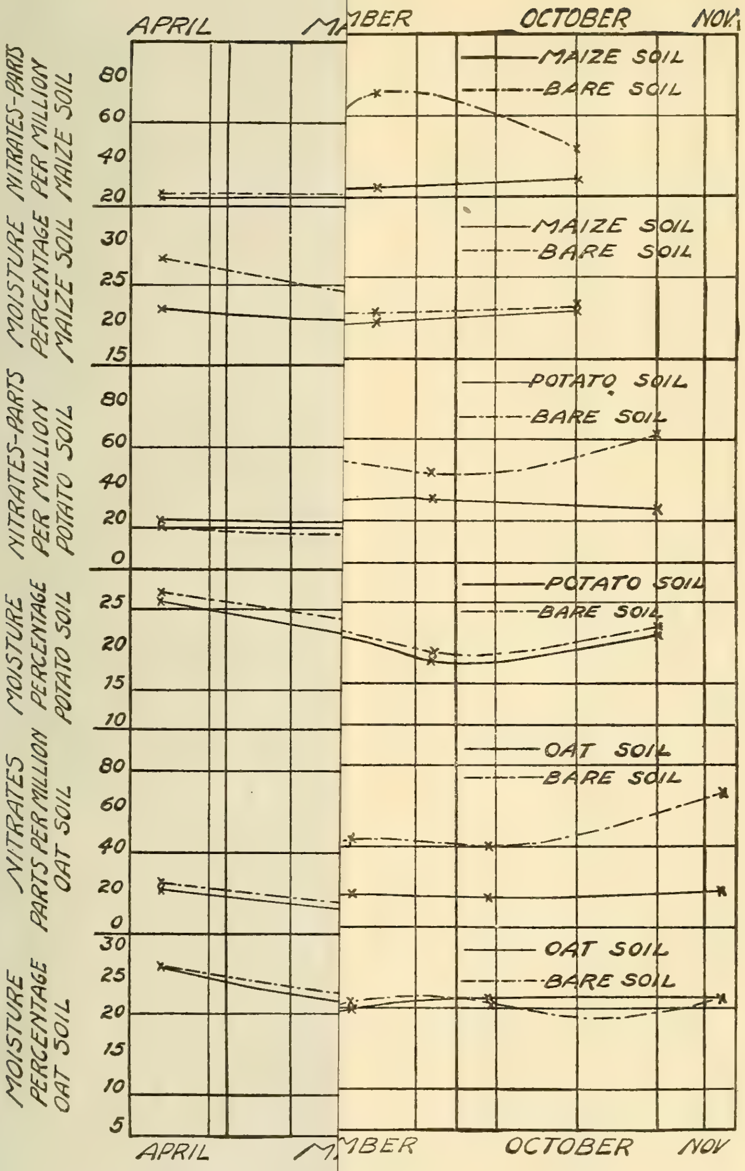


DIAGRAM XVI.— *Nitrates and moisture in surface ten inches of soil planted to corn (maize), potatoes, and oats and in bare soil, 1912, also mean daily air temperature and daily rainfall for the growing season*



DIA*re soil, 1911*

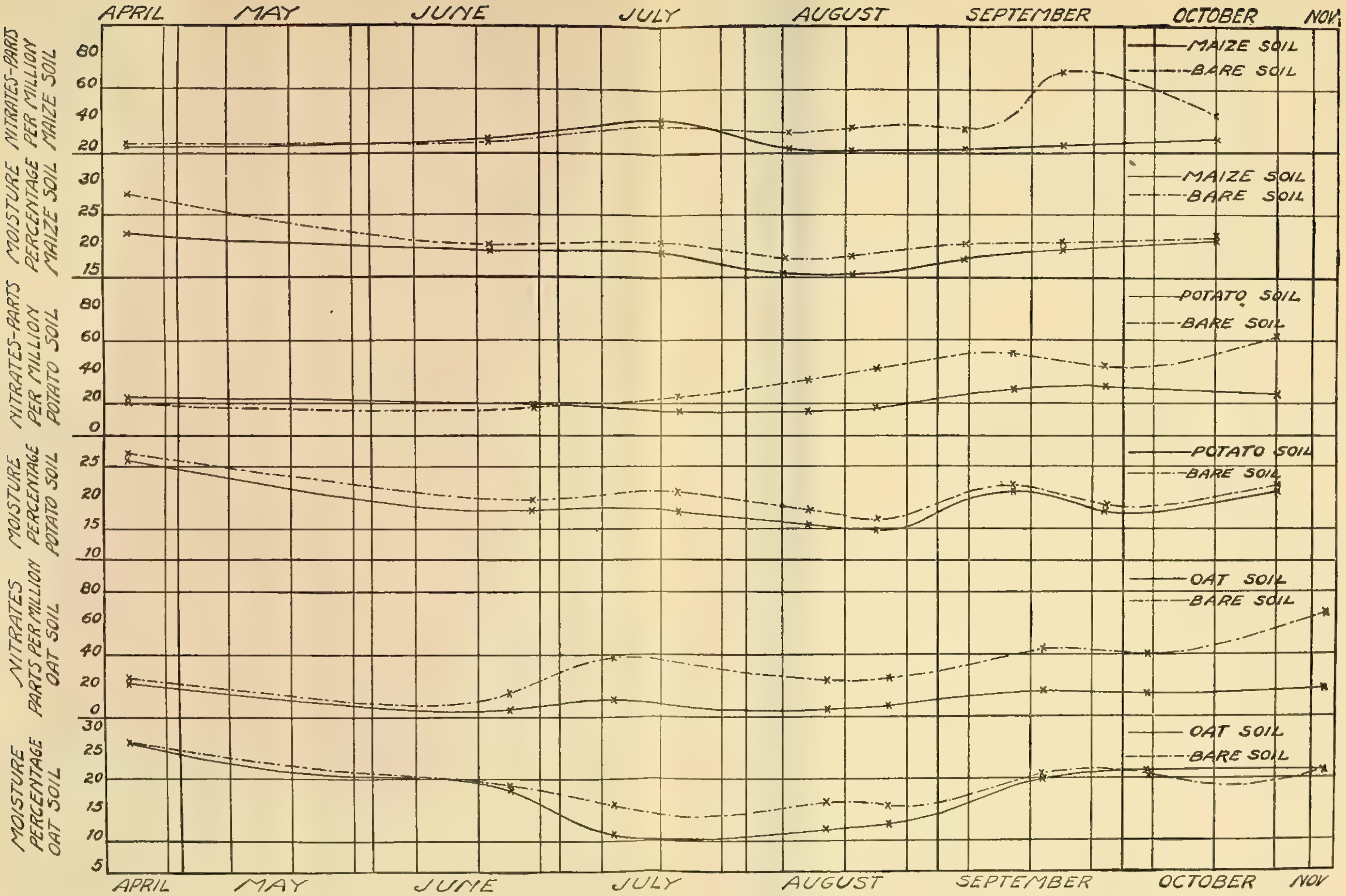


DIAGRAM VII.— *Nitrates and moisture in second eight inches of soil planted to maize, potatoes, and oats and in bare soil, 1911* crops and treatments to the nitrate content of the soil. It will be remembered that the analyses of April 19 to 26 were made before the crops were planted. These data for the surface eight inches, for the second eight inches, and for the average of these two depths, are given in Table 19:

TABLE 19. RATIO OF NITRATES IN PLANTED SECTIONS OF PLATS TO NITRATES IN UNPLANTED SECTIONS

First eight inches

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209, 2401, 2409	Maize, mulched.....	96	113	75	61	37	36	44	94
2202, 2210, 2402, 2410	Maize, scraped......	139	102	77	108	54	43	40	16
2203, 2211, 2403, 2411	Maize, cultivated....	132	105	75	56	37	27	16	34
2204, 2212, 2404, 2412	Maize, harvested August 1...........	88	90	93	69	76	73	85	61
2205, 2213, 2405, 2413	Maize, planted June 26, harvested August 1............	84	85	84	104	83	86	72	89
2206, 2214, 2406, 2414	Potatoes...........	118	75	57	35	32	31	42	28
2207, 2215, 2407, 2415	Oats...............	72	8	19	11	14	19	23	20
2208, 2216, 2408, 2416	Oats, followed by maize............	100	10	7	8	17	11	13	17

Second eight inches

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209, 2401, 2409	Maize, mulched.....	116	112	76	76	44	48	32	57
2202, 2210, 2402, 2410	Maize, scraped......	107	100	68	95	56	50	52	43
2203, 2211, 2403, 2411	Maize, cultivated....	100	108	108	67	60	61	35	67
2204, 2212, 2404, 2412	Maize, harvested August 1...........	132	100	74	90	72	86	69	78
2205, 2213, 2405, 2413	Maize, planted June 26, harvested August 1............	91	94	87	89	90	69	70	107
2206, 2214, 2406, 2414	Potatoes...........	100	94	60	46	40	55	70	44
2207, 2215, 2407, 2415	Oats...............	80	27	13	23	29	37	41	26
2208, 2216, 2408, 2416	Oats, followed by maize............	73	37	14	14	25	27	33	36

TABLE 19 (*continued*)

(See tables 1, 5, 6, 7, 8)

Average of first and second eight inches

Plat	Crop	April 19 to 26	June 16 to 27	July 10 to 21	August 4 to 14	August 16 to 24	August 31 to September 18	September 20 to October 5	October 9 to November 4
2201, 2209, 2401, 2409	Maize, mulched	104	112	75	65	40	45	37	75
2202, 2210, 2402, 2410	Maize, scraped	128	100	72	106	54	44	43	21
2203, 2211, 2403, 2411	Maize, cultivated	113	105	83	57	41	32	20	39
2204, 2212, 2404, 2412	Maize, harvested August 1	104	93	88	72	74	76	80	65
2205, 2213, 2405, 2413	Maize, planted June 26, harvested August 1	85	86	85	102	85	82	71	92
2206, 2214, 2406, 2414	Potatoes	107	81	58	37	35	36	48	32
2207, 2215, 2407, 2415	Oats	75	16	15	12	18	23	27	22
2208, 2216, 2408, 2416	Oats, followed by maize	85	16	8	8	19	15	17	23

The analyses of April 19 to 26 were all made before any of the crops were planted, and therefore they represent the natural relation of the respective sections of the plats when uninfluenced by plants. In the maize plats there is a tendency for the nitrates under the crop to increase sometime during the growing season in the scraped plat and in the two plats harvested August 1. The other two maize plats do not show this tendency.

As has been remarked before, there appears to be a relation between the size of the crop and the tendency of the nitrates to increase during the active growing season. It is impossible to compare the yields on the early-harvested plats with those on the other plats, but of the late-harvested plats those that increased in nitrates during the early part of the growing season were the ones that yielded least — namely, the scraped plats 2202, 2210, 2402, 2410. The yields of these plats are stated in Table 20:

TABLE 20. YIELDS OF MAIZE (GRAIN AND FODDER) ON PLATS HARVESTED AT MATURITY

Plat	Treatment	Weight of crop (grams per plat)	Nitrogen in crop (grams per plat)
2201, 2209, 2401, 2409	Mulched	16,457	123
2202, 2210, 2402, 2410	Scraped	13,369	98
2203, 2211, 2403, 2411	Cultivated	17,425	135

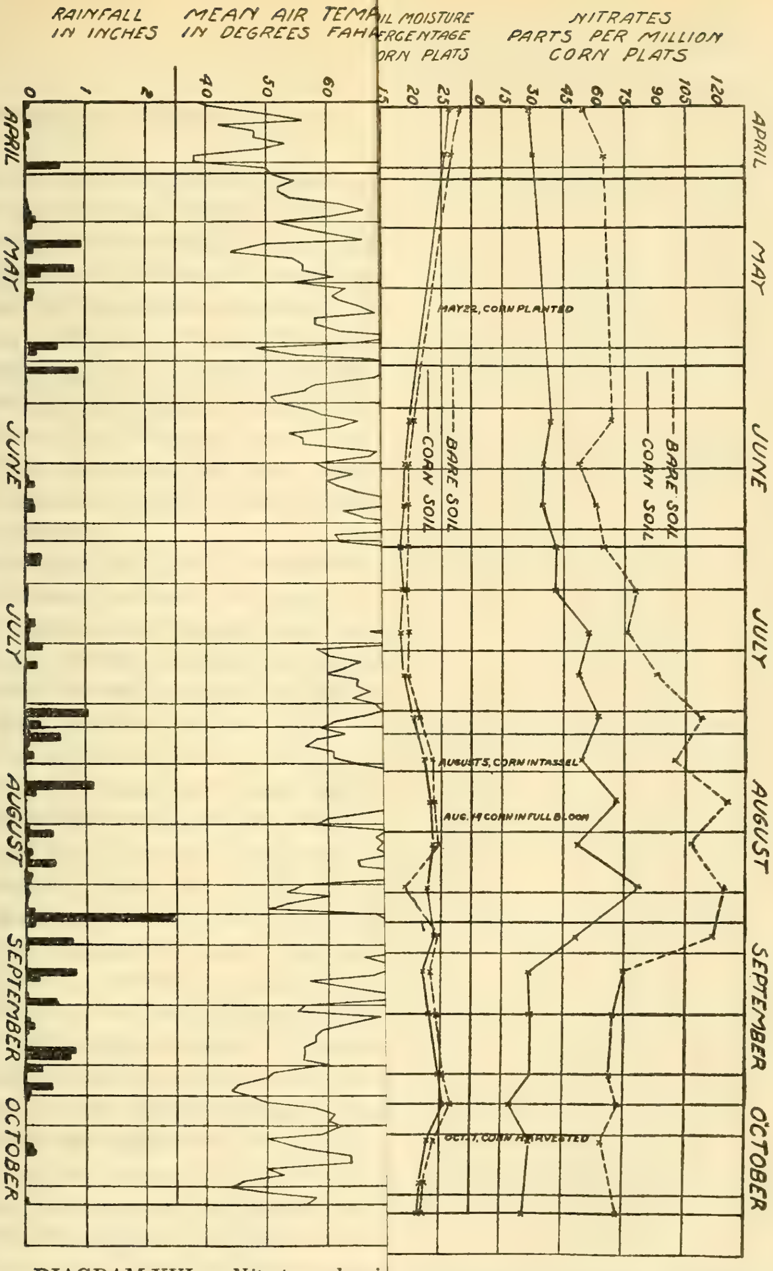


DIAGRAM XVI.— *Nitrates and mois* *mean daily air temperature and daily*

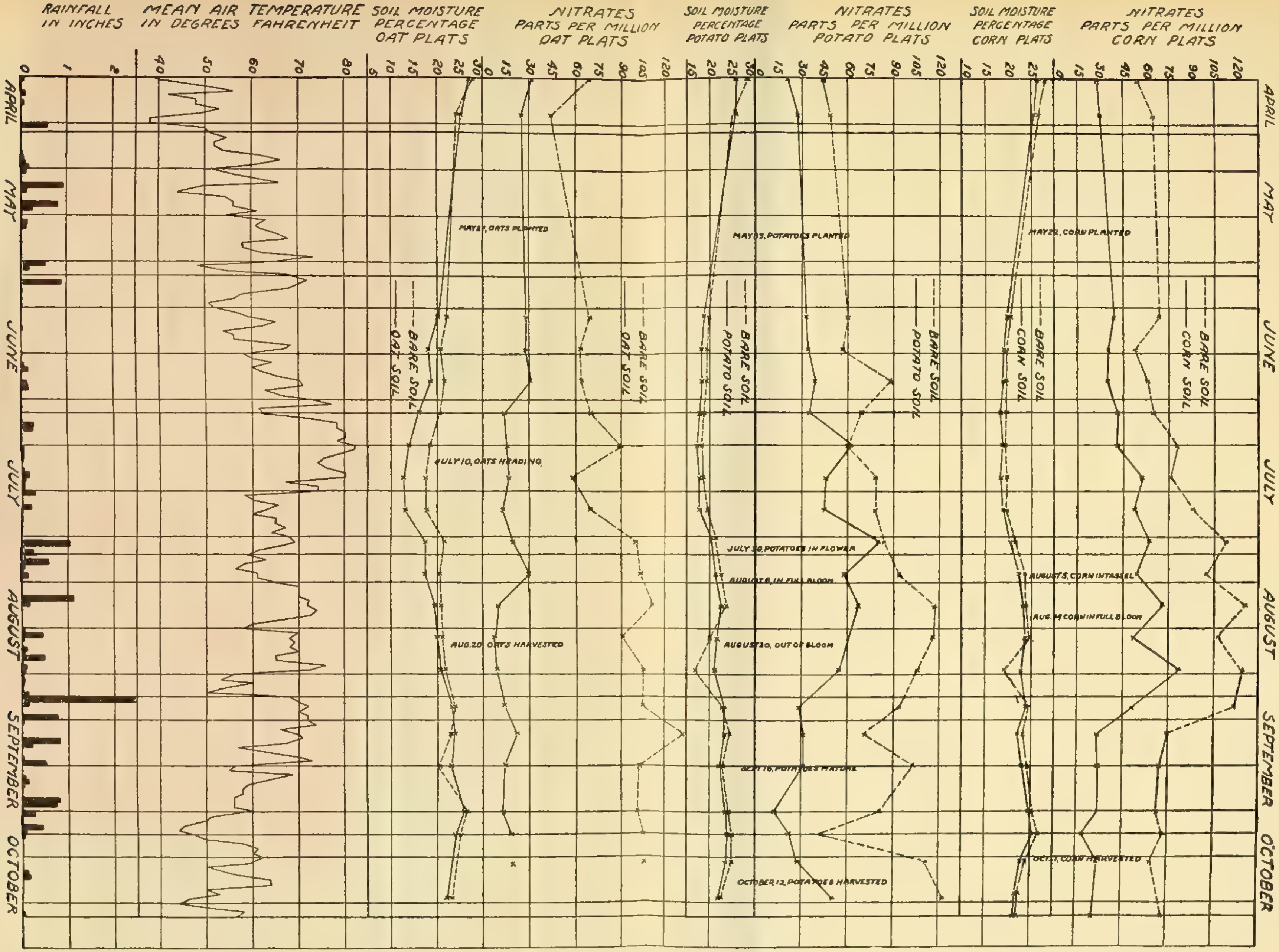


DIAGRAM XVI.— *Nitrates and moisture in surface ten inches of soil planted to corn (maize), potatoes, and oats and in bare soil, 1912, also mean daily air temperature and daily rainfall for the growing season*

Both the yield of plant substance and the nitrogen in the crop are least on the scraped plats, which are the ones that exhibit a tendency for the nitrates to increase during the growing period. This would indicate that the utilization of nitrates is greater on the plats producing larger yields; but that the high nitrate content of the maize plats cannot be due entirely to this is evidenced by the fact that the nitrates under the plants are higher in August than are the nitrates in the unplanted soil.

During the later stages of growth the maize plats harvested at maturity all decrease in nitrates at about the same rate. In this respect they differ from the plats that were harvested August 1, which maintain a higher nitrate content during the latter part of the season as has already been mentioned.

The marked falling-off in the nitrates after the middle of August on the plats of maize harvested at maturity as compared with the high nitrate content earlier in the season is not in keeping with the nitrogen absorption by the crop during the early and the later part of the season. Yields and analyses of the maize on these plats showed that by August 1 the maize plants had absorbed 44 per cent of all their nitrogen. During the early period the nitrate content of the planted soil was increasing nearly as rapidly as, and in some cases more rapidly than, that of the unplanted soil. After the middle of August, by which time fully 50 per cent of the nitrogen must have been absorbed, the nitrate content of the planted soil decreased very markedly while the nitrate content of the unplanted soil increased. If the decreased nitrate content in the planted soil were caused by nitrogen absorption exclusively, there would be a tendency for the nitrates in the planted soil to increase as do the nitrates in the unplanted soil; but in general there is no such tendency even late in the season, when nitrogen absorption must have ceased. This again indicates a depressing influence of the plant on nitrification during the later stages of growth.

Comparing the oat plats planted to maize after the oats were harvested with those kept unplanted, it will be seen that there is a tendency for the nitrates to run higher in the latter. This indicates that the growth of the young maize plants has stimulated the formation of nitrates and has overcome the depressing influence of the mature oat plants.

In diagrams VIII to XV these relations of nitrates under the plants to those in the unplanted soil of the corresponding plats are shown graphically.

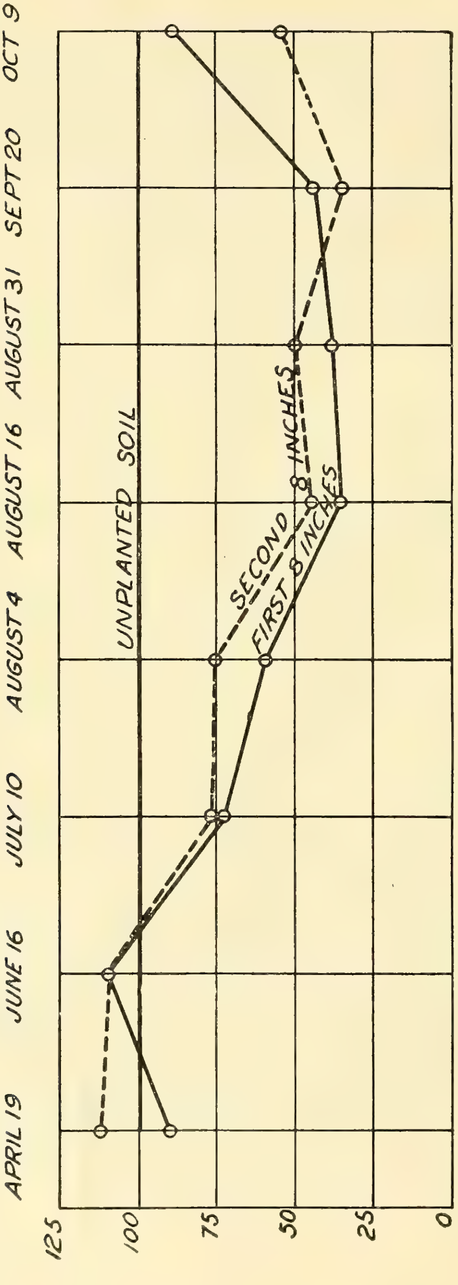


DIAGRAM VIII.— *Relative nitrate content of soil under maize mulched, 1911*

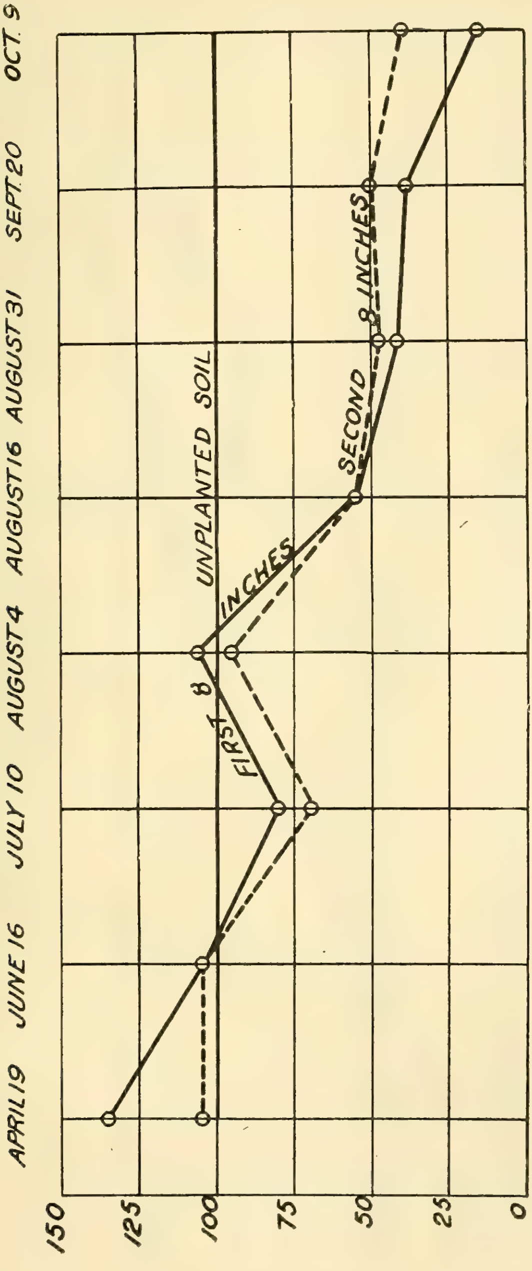


DIAGRAM IX.— *Relative nitrate content of soil under maize scraped, 1911*

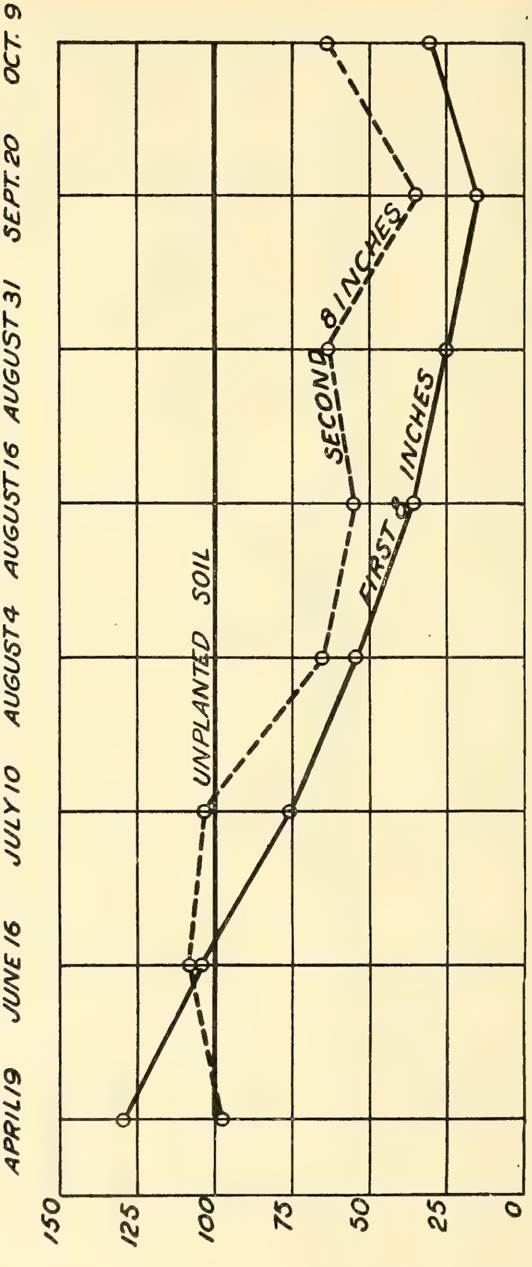


DIAGRAM X.— *Relative nitrate content of soil under maize cultivated, 1911*

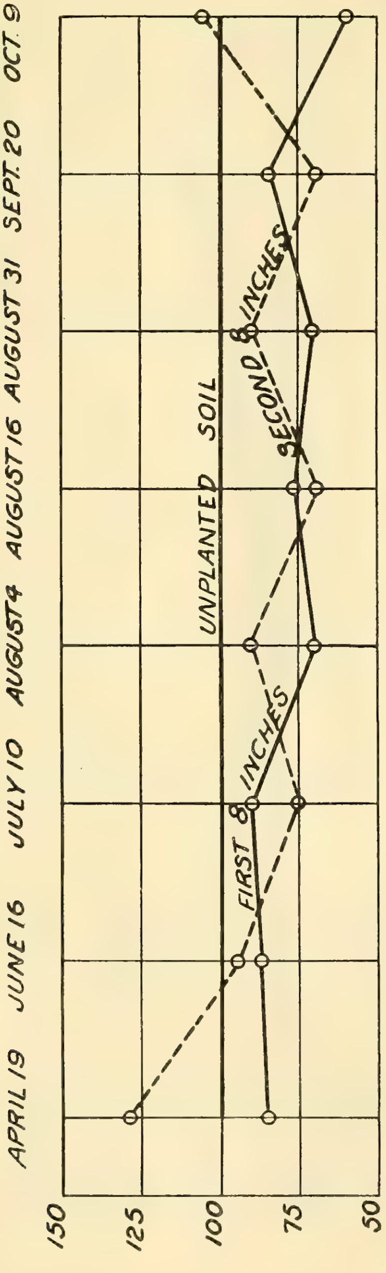


DIAGRAM XI.—*Relative nitrate content of soil under maize harvested August 1 and cultivated after harvest, 1911*

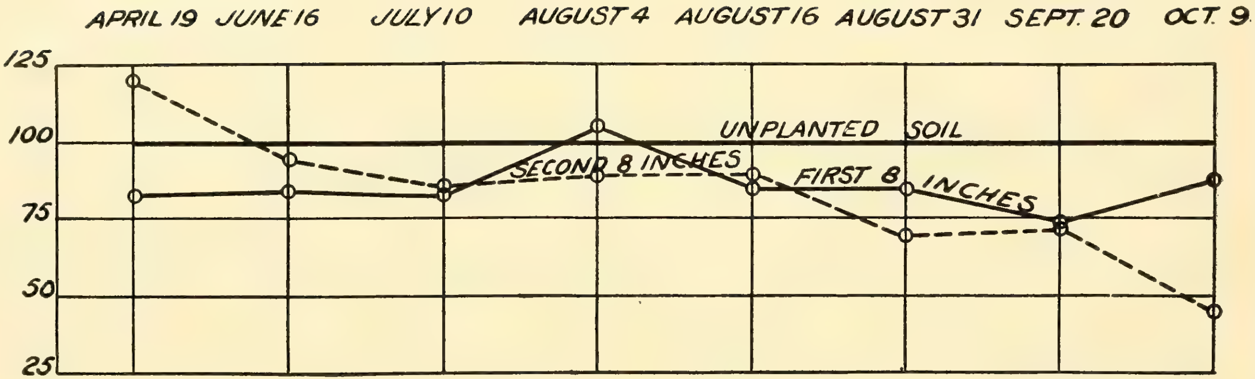


DIAGRAM XII.—*Relative nitrate content of soil under maize planted June 26, harvested August 1, and cultivated after harvest, 1911*

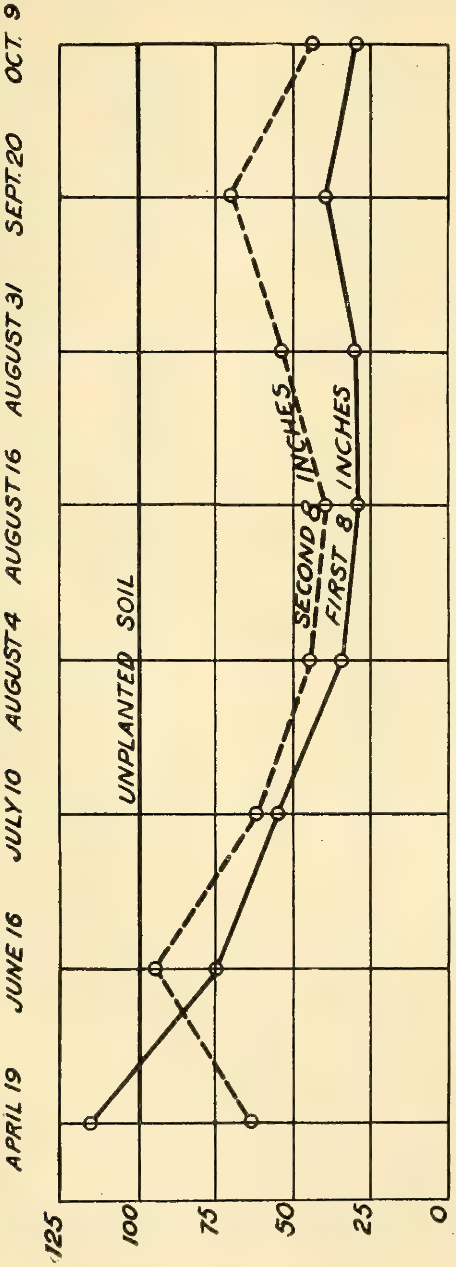


DIAGRAM XIII.—*Relative nitrate content of soil under potatoes, 1911*

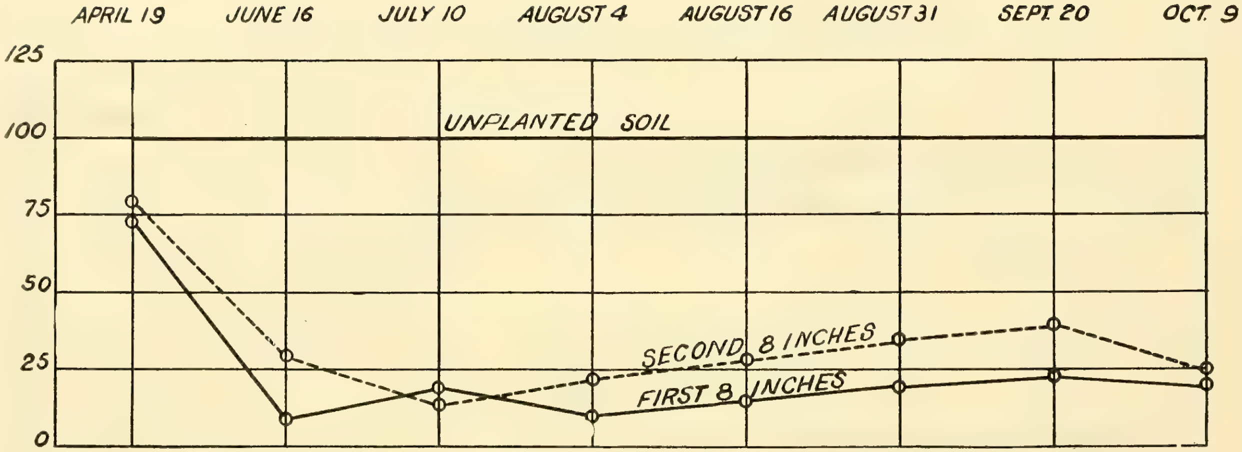


DIAGRAM XIV.— *Relative nitrate content of soil under oats cultivated after harvest, 1911*

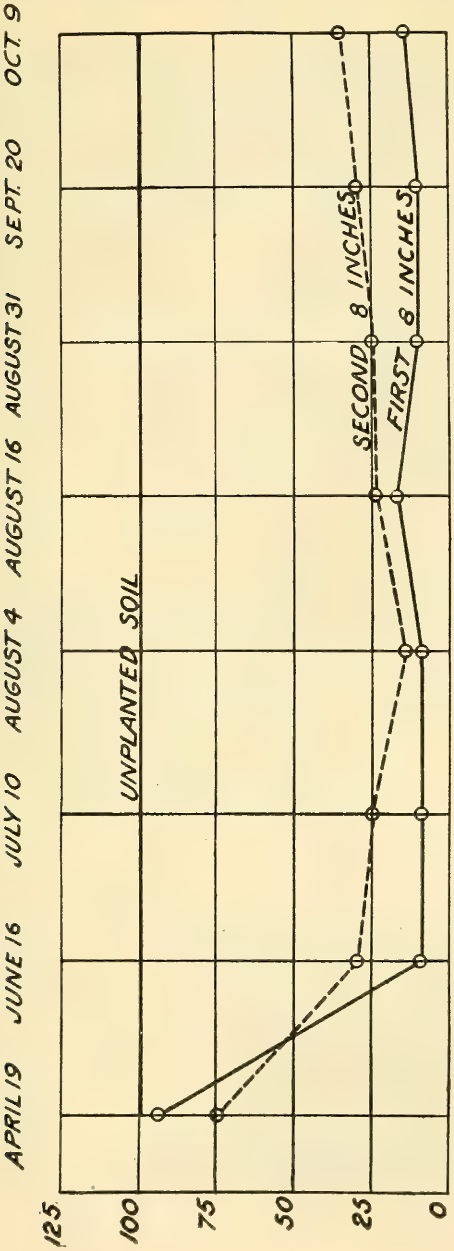


DIAGRAM XV.—*Relative nitrate content of soil under oats followed by maize, 1911*

Experiments in 1912

The plats used in 1911 were planted in 1912 to the same kinds of crops that were grown on them in the previous year. It was thought that this might give a cumulative effect of the crop on the soil. As before, the middle sections of the plats were not planted. In one respect the plats were different from those used in 1910 and 1911, in which cases the entire plats had been planted to some crop in the preceding years. The middle sections had therefore been fallowed for two years. As a result of the fallow of the previous year the nitrates in the middle sections were much higher in spring than those in the planted sections, except in the mulched maize plats where the difference was not great.

The arrangement of the plats and their treatment, as given below, differed only in time of planting and harvesting from that of 1911:

Plat	Crop and soil treatment
2201, 2209, 2401, 2409	Maize planted May 22. Mulched
2202, 2210, 2402, 2410	Maize planted May 22. Scraped
2203, 2211, 2403, 2411	Maize planted May 22. Cultivated all summer
2204, 2212, 2404, 2412	Maize planted May 22, harvested August 20, and allowed to regrow. Cultivated all summer
2205, 2213, 2405, 2413	Maize planted July 5, harvested August 20. Cultivated all summer
2206, 2214, 2406, 2414	Potatoes planted May 23. Cultivated
2207, 2215, 2407, 2415	Oats planted May 21. Cultivated after harvest
2208, 2216, 2408, 2416	Oats planted May 21, followed by maize. Cultivated the maize

The temperature during the growing season was somewhat below normal. The first half of July was the warmest part of the summer. Rainfall was deficient in June and July and the yield of oats was lessened by the drought. Growth continued later than usual in the autumn because of the warm, moist weather in September. Potatoes were fully mature about the middle of September and maize was harvested on October 20, except on the early-harvested plats.

No manure nor fertilizer was applied to the soil. The growth of all crops was normal but the yields were not large.

TABLE 21. NITRATES IN SOIL OF PLANTED AND UNPLANTED SECTIONS OF PLATS, 1912. SURFACE TEN INCHES (PARTS PER MILLION)

Plat	Crop	April 19	April 27	June 10	June 17	June 24	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26	September 3	September 9	September 16	September 26	October 1	October 7	October 18
2201, 2209, 2401, 2409	Maize, mulched	19	17	40	26	30	37	37	50	60	58	60	51	47	47	24	17	...	22	13	25	26
2201, 2209, 2401, 2409	None	22	23	43	28	38	32	39	42	56	64	53	52	55	76	52	29	...	34	32	30	36
2202, 2210, 2402, 2410	Maize, scraped	27	34	42	30	29	33	45	48	63	60	46	70	54	63	39	47	38	27	26	36	34
2202, 2210, 2402, 2410	None	66	68	67	65	54	53	53	74	85	103	79	100	89	93	47	55	57	69	104	70	79
2203, 2211, 2403, 2411	Maize, cultivated	28	30	39	36	34	41	41	57	52	62	54	71	50	84	50	29	29	29	19	30	26
2203, 2211, 2403, 2411	None	54	65	69	52	60	64	80	76	90	113	101	125	107	123	119	74	69	68	72	64	73
2204, 2212, 2404, 2412	Maize, harvested August 20	47	39	50	42	47	45	53	54	78	65	71	81	75	93	70	53	48	60	103	58	76
2204, 2212, 2404, 2412	None	60	62	64	48	56	56	68	87	87	120	122	110	101	116	100	89	75	49	85	98	111
2205, 2213, 2405, 2413	Maize, planted July 5, harvested August 20	59	60	52	50	68	65	73	67	83	86	109	118	104	111	86	90	84	102	73	84	83
2205, 2213, 2405, 2413	None	78	69	52	57	61	90	69	70	109	104	103	153	103	135	114	104	91	103	90	106	106
2206, 2214, 2406, 2414	Potatoes	22	29	33	35	39	35	62	46	45	82	58	66	61	55	29	31	31	14	24	28	53
2206, 2214, 2406, 2414	None	45	49	60	57	88	69	62	78	79	83	95	118	116	105	94	71	104	83	43	111	124
2207, 2215, 2407, 2415	Oats	31	26	28	27	31	13	15	17	12	19	31	10	8	9	14	22	15	13	20	...	...
2207, 2215, 2407, 2415	None	70	44	69	64	64	70	90	58	70	99	103	112	92	105	104	134	102	102	105	...	...
2208, 2216, 2408, 2416	Oats, followed by maize	25	22	30	30	26	20	11	13	10	18	9	10	7	11	13	17	14	16	34	...	...
2208, 2216, 2408, 2416	None	77	54	52	47	60	43	46	63	61	58	86	84	81	80	71	76	96	95	72	...	...

TABLE 22. MOISTURE IN PLANTED AND UNPLANTED SECTIONS OF PLATS, 1912. SURFACE TEN INCHES (PERCENTAGE OF DRY SOIL)

Plat	Crop	April 19	April 27	June 10	June 17	June 24	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26	September 3	September 9	September 16	September 26	October 1	October 7	October 18
2201, 2209, 2401, 2409	Maize, mulched	28.1	25.5	22.6	20.7	20.6	21.0	20.6	20.1	22.7	21.8	25.6	25.8	25.9	25.7	26.5	25.8	26.2	27.8	27.0	26.6	25.3
2201, 2209, 2401, 2409	None	27.9	23.9	22.4	20.4	20.3	21.5	20.8	20.0	21.2	22.3	26.4	25.5	26.3	26.3	23.3	26.4	27.6	26.8	26.9	27.0	24.8
2202, 2210, 2402, 2410	Maize, scraped	26.1	25.8	21.7	19.5	19.4	18.2	17.3	16.7	18.4	17.2	21.1	22.5	20.5	21.8	24.2	23.2	24.3	25.6	25.4	24.0	22.4
2202, 2210, 2402, 2410	None	26.7	26.9	20.1	19.2	18.8	18.3	17.2	17.1	19.2	18.6	21.9	23.4	21.3	25.9	24.0	23.9	25.1	25.2	25.6	24.4	22.9
2203, 2211, 2403, 2411	Maize, cultivated	26.7	25.9	20.3	18.9	19.0	18.6	18.9	18.6	19.1	21.1	22.7	24.4	24.3	22.9	24.0	22.5	23.8	24.9	25.6	23.6	22.2
2203, 2211, 2403, 2411	None	28.1	26.6	20.7	19.6	19.2	19.5	18.9	19.9	19.4	21.5	23.6	24.1	24.7	18.9	24.5	23.4	24.4	25.0	26.7	23.9	22.8
2204, 2212, 2404, 2412	Maize, harvested August 20	25.2	24.4	19.4	18.8	19.2	18.7	18.1	18.7	18.9	20.5	22.5	23.1	23.1	23.1	23.3	22.2	22.8	24.7	25.0	23.8	21.3
2204, 2212, 2404, 2412	None	26.4	24.5	19.3	18.4	19.1	18.7	18.0	18.5	18.9	20.9	22.8	23.8	24.1	23.2	24.0	22.4	23.4	24.9	25.6	23.6	21.8
2205, 2213, 2405, 2413	Maize, planted July 5, harvested August 20	24.7	24.2	19.8	18.0	18.6	18.2	17.9	18.2	18.6	21.3	21.7	23.2	22.2	22.7	22.9	23.3	22.8	24.4	24.8	24.4	21.7
2205, 2213, 2405, 2413	None	25.0	24.5	20.9	18.9	18.9	18.9	18.1	18.7	18.8	21.9	22.1	23.6	22.8	17.6	23.9	23.3	22.5	24.8	24.6	24.5	23.4
2206, 2214, 2406, 2414	Potatoes	26.7	25.9	19.4	18.4	18.8	18.2	17.4	17.9	17.9	20.6	21.4	22.8	21.7	21.4	23.0	23.4	22.5	24.3	24.8	24.0	22.3
2206, 2214, 2406, 2414	None	28.6	25.2	20.0	19.5	19.5	18.8	18.7	18.8	19.5	21.4	22.1	23.7	20.3	17.2	23.0	24.4	22.9	24.7	25.0	25.0	22.7
2207, 2215, 2407, 2415	Oats	27.0	24.9	20.2	18.0	18.6	15.9	13.9	12.8	12.9	17.4	17.4	19.8	20.0	21.1	23.6	23.8	23.1	26.0	24.4	....	....
2207, 2215, 2407, 2415	None	27.4	24.5	22.0	20.7	21.8	20.4	18.1	17.8	17.6	21.5	20.5	20.8	21.0	21.8	23.7	23.3	20.3	26.2	24.7	....	....
2208, 2216, 2408, 2416	Oats, followed by maize	27.4	24.8	20.6	18.4	18.9	15.8	13.6	12.2	12.2	17.7	17.3	19.7	20.1	21.4	23.6	22.8	22.8	25.9	24.1	....	....
2208, 2216, 2408, 2416	None	27.7	25.4	21.6	20.8	21.3	20.0	18.8	18.1	17.7	20.9	21.1	21.7	21.7	21.6	23.0	22.6	23.1	26.0	23.0	....	....

TABLE 23. RATIO OF NITRATES IN PLANTED SECTIONS OF PLATS TO NITRATES IN UNPLANTED SECTIONS

Plat	Crop	April 19	April 27	June 10	June 17	June 24	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26	September 3	September 9	September 16	September 26	October 1	October 7	October 18
2201, 2209, 2401, 2409..	Maize, mulched............	86	74	93	93	79	116	95	119	107	91	113	98	85	62	46	59	...	65	41	83	72
2202, 2210, 2402, 2410..	Maize, scraped............	41	50	63	46	54	62	85	65	74	58	58	70	61	68	83	85	67	39	25	51	43
2203, 2211, 2403, 2411..	Maize, cultivated..........	52	46	57	69	57	64	51	75	58	55	53	57	47	68	42	39	42	43	26	47	36
2204, 2212, 2404, 2412..	Maize, harvested August 20..	78	63	78	87	84	80	78	62	90	54	58	74	74	80	70	60	64	122	121	59	68
2205, 2213, 2405, 2413..	Maize, planted July 5, harvested August 20.........	76	87	100	88	111	72	106	96	76	83	106	77	101	82	75	87	92	99	81	79	78
2206, 2214, 2406, 2414..	Potatoes..................	49	59	55	61	44	51	100	59	57	99	61	56	53	52	30	44	30	17	56	25	43
2207, 2215, 2407, 2415..	Oats......................	44	59	41	42	48	19	17	29	17	19	30	9	9	9	13	16	15	13	19	...	...
2208, 2216, 2408, 2416..	Oats, followed by maize.....	32	41	58	64	43	46	24	21	16	31	10	12	9	14	18	22	15	17	47	...	...

Soil samples for moisture and nitrates were taken to a depth of ten inches except on the cultivated plats, on which the surface two inches was removed before boring and the samples were taken to a depth of ten inches under the layer removed. The reason for removing the surface two inches on these plats was to eliminate from the samples the loose, dry layer, in which nitrate formation would not be expected to proceed under the same influences that operate in the moist soil below. In Diagram XVI is shown the course of the nitrates under the cultivated maize and under potatoes and oats.

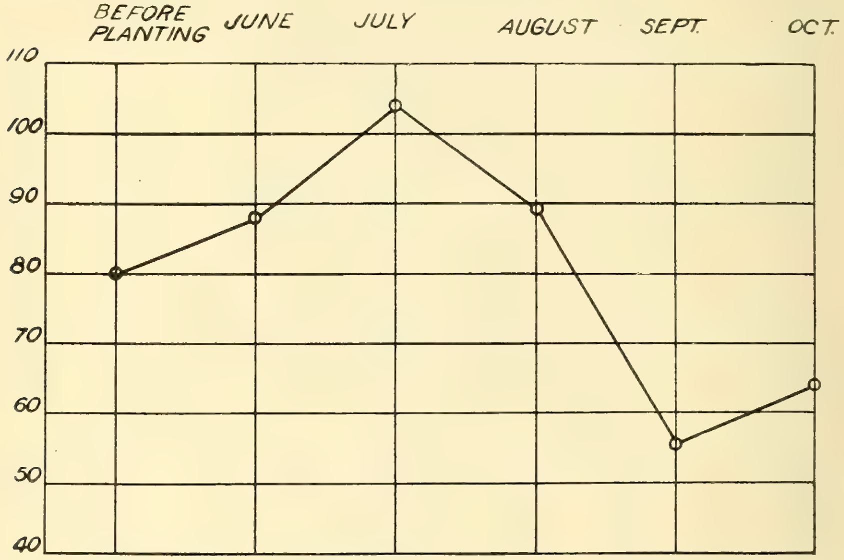


DIAGRAM XVII.— *Relative nitrate content of soil under maize mulched, 1912*

The nitrate content and the moisture content of the soil on the planted and unplanted sections of all plats are shown in tables 21 and 22. The characteristic relationship between the crop and the nitrate content of the soil is again apparent in the former table, nitrates being higher under maize than under potatoes during the active growing period and being lowest under oats.

Nitrates are sometimes higher in the soil under maize than in the unplanted soil on the mulched plats; but the nitrates are so high in the unplanted soil of the other maize plats, owing to the fallow of the previous year, that only on the late-planted plats are the nitrates at any time actually higher under the crop than on the bare soil.

The relations of the crops to nitrates are well brought out in Table 23 and in diagrams XVII to XXIV. The figures on which these diagrams are based have been obtained by considering the nitrates in the bare spaces as 100 and calculating, on that basis, the nitrates in the planted sections of the plats. These percentages are then grouped into periods embracing all the analyses before planting, and the analyses after planting by months or such parts of months as elapsed before harvest.

The mulched maize plats, represented in Diagram XVII, show a maximum nitrate content in July, at which time the nitrates were higher under the maize than in the bare soil. After that there is a decrease to September and a slight increase in October.

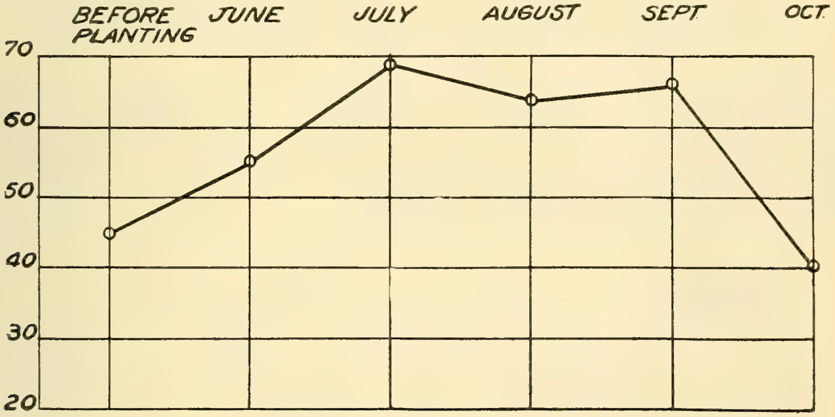


DIAGRAM XVIII.—*Relative nitrate content of soil under maize scraped, 1912*

The scraped maize plats, represented in Diagram XVIII, show a maximum nitrate content in July, but it is not so high under maize as in the unplanted soil. There is a decrease after July.

The cultivated maize plats, represented in Diagram XIX, correspond in the main with the previous plats. All these maize plats exhibit the characteristic stimulation of nitrate formation in midsummer and partial inhibition in late summer and in the fall.

The maize plats planted on May 22 and harvested on August 20 are represented in Diagram XX. The maize on these plats was allowed to regrow after harvest; in this respect the treatment differed from that of the previous year. The August figures are based on the analyses made previous to August 20. In September and October there is an increase in the nitrate content of the soil. If the regrowth of maize has a stimulating effect on nitrate formation this increase is to be expected, as the maize made a regrowth of about eighteen inches.

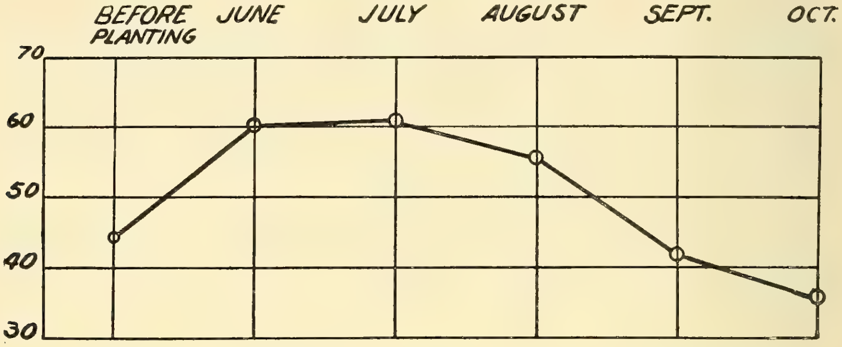


DIAGRAM XIX.— *Relative nitrate content of soil under maize cultivated, 1912*

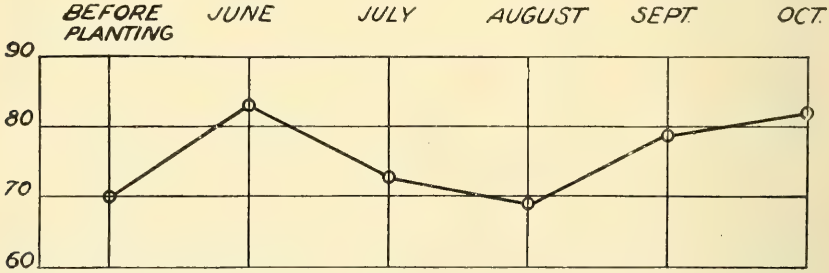


DIAGRAM XX.— *Relative nitrate content of soil under maize harvested August 20 and allowed to regrow, 1912*

It will be noted that this was the only set of maize plats in which any marked increase of nitrates occurred. The increase of nitrates under maize at this time of year and coincident with the regrowth, is evidence that the time of year is not the only determining factor in the formation of nitrates; nor is the decrease of nitrates during the later stages of growth due entirely to the removal of easily nitrifiable organic matter, otherwise the nitrate content would not have risen in the soil on which a regrowth of maize occurred.

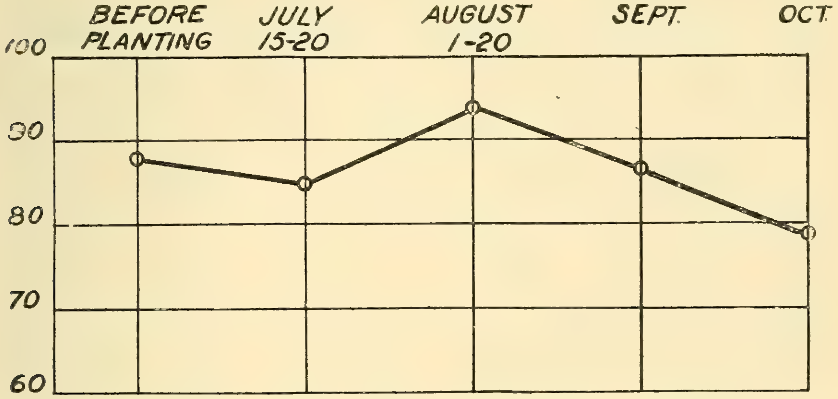


DIAGRAM XXI.—*Relative nitrate content of soil under maize planted July 5, harvested August 20, 1912*

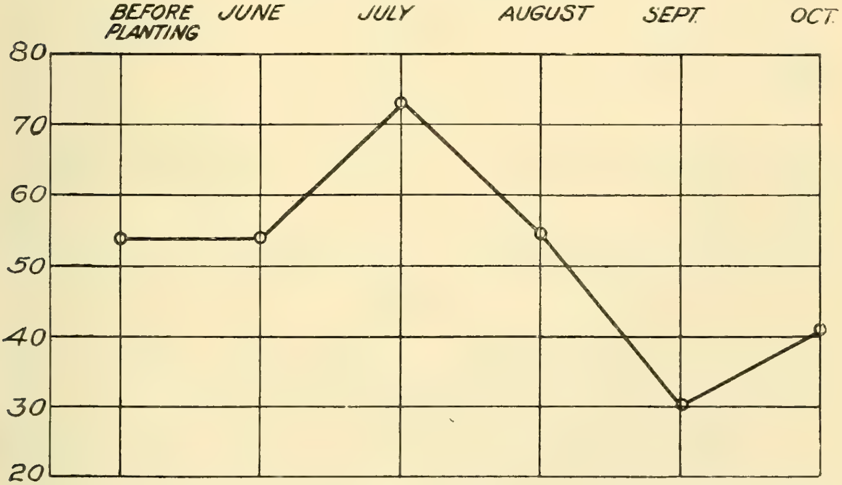


DIAGRAM XXII.—*Relative nitrate content of soil under potatoes, 1912*

Nitrates in the late-planted maize plats are shown in Diagram XXI. Owing to the late planting — July 5 — the nitrates never fall very low and are highest in August; this is another indication that the time of year is not the controlling factor in the maximum nitrate content. Following the harvest of this maize on August 20 there is a decrease in nitrates.

The nitrates under potatoes are shown in Diagram XXII. As in previous years the maximum nitrate content occurs in July, and the nitrates decrease from that time until the close of the season.

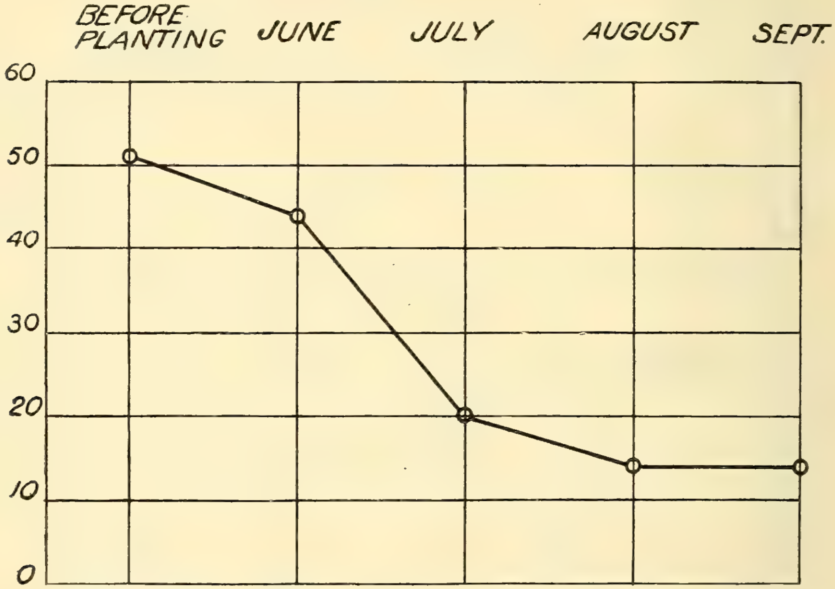


DIAGRAM XXIII.— *Relative nitrate content of soil under oats, cultivated after harvest, 1912*

The oat plats, represented in diagrams XXIII and XXIV, show the characteristic decline in nitrates, except in the plats on which maize was planted after the oats were harvested. In these plats there was a slight rise of nitrates in September; but, since the maize grew only five or six inches high, the effect on the nitrates was unimportant.

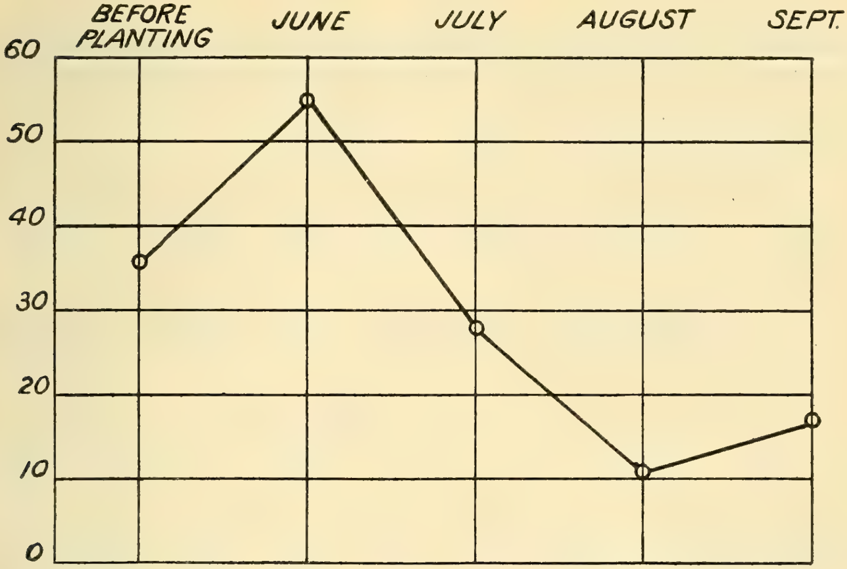


DIAGRAM XXIV.— *Relative nitrate content of soil under oats followed by maize, 1912*

The relation of the nitrate content of the soil to the quantities of nutrients removed by crops

It has been suggested by a number of investigators that the bacterial activity of the soil is dependent on the supply of readily soluble mineral nutrients that it contains. Assuming this to be true, the question arises whether a certain crop might influence the production of nitrates in the soil by removing a greater or less quantity of the soluble mineral nutrients.

As maize and oats have been grown in this experiment for a number of years, it is possible to make some comparisons between these two plants in regard to the quantities of nutrients removed and to the nitrate content of the soil during the latter part of the season, when the removal of the nutrient substances may be expected to affect the nitrate content.

The crops removed from the plats used in these experiments were all sampled and the total nitrogen was determined. Unfortunately, the ash was not analyzed, therefore no exact figures are obtainable for the mineral constituents; but, since the variation in yields between the two crops, maize and oats, was very large, probably the error introduced by employing an average figure for the percentage of mineral constituents would be insignificant as compared with the differences in the quantities removed in the crops. The percentages of phosphoric acid (P_2O_5) and potash (K_2O) given in the Yearbook of the Department of Agriculture for 1894 (pages 565 to 568) have been used.

In Table 24 are given the relative quantities of nitrogen, phosphoric acid, and potash removed in the maize and oat crops, and the relative nitrate contents of the plats at the time of harvest or as near that date as is practicable:

TABLE 24. RELATIVE QUANTITIES OF NUTRIENTS REMOVED BY MAIZE AND OATS AND RELATIVE NITRATE CONTENTS OF THE SOIL AT HARVEST

	1908 Maize	1909 Oats	1910	
			Maize	Oats
Relative yields of				
Dry matter	100	72	100	755
Nitrogen	100	71	100	828
Phosphoric acid	100	91	100	1,523
Potash	100	176	100	1,265
Dates of nitrate determinations	Sept. 17	Aug. 7	Oct. 17	Aug. 17
Relative nitrate contents of soil	100	7	100	34

It will be noticed that in 1908 and 1909 the oat crop was small as compared with the maize crop, while in 1910 it was relatively very large. In both instances the nitrate content of the oat soil was characteristically much lower than that of the maize soil. The characteristic relation of nitrate content under oats and under maize appears to be independent of the quantities of nutrients removed by the crops.

The influence of alfalfa and of timothy on the production of nitrates in soil

On the experiment field in which most of the experiments just described were conducted, and consequently on similar soil, certain plats had been seeded to alfalfa and contiguous plats to timothy in the spring of 1906. These plats remained continuously in the same crops until the fall of 1911. At the time when the seeding was done, part of the land planted to each of the crops received an application of quicklime at the rate of 2000 pounds per acre. Alfalfa roots on both limed and unlimed soil possessed tubercles. In the spring of 1910 strips of land through both the limed and the unlimed parts of the alfalfa and the timothy plats were hoed bare of vegetation, and these strips were kept free from plants of all kinds during 1910 and 1911.

The nitrate-producing power of alfalfa soil and of timothy soil

The rate at which nitrate formation takes place in soil is well known to be influenced by a number of conditions, such as the basicity of the soil, the degree of aeration, and the like. That it may be influenced by the nature of the vegetation growing on the soil appears to be true of the soil used in these experiments. The method adopted for measuring nitrate production was as follows: The soil was sampled by means of a soil auger to a depth of eight inches. The borings from the plat or section of plat to be tested were mixed, quartered, and placed in an air-tight jar while still moist. When the jar was brought to the laboratory, moisture and nitrates were determined within twelve hours after sampling. Nitrates were determined in 100 grams of moist soil by means of the disulfonic-acid method. Another 100 grams of moist soil was placed in a bottle having a capacity of 250 cubic centimeters, and sufficient water was added to bring the moisture content to 25 per cent of the dry weight of the soil. A loose cotton plug was inserted into the mouth of the bottle, which was then placed in an incubator and kept at a temperature of 30° C. for the number of days stated in the following tables. In some of the earlier tests, 500 milligrams of ammonium sulfate was added to each 100 grams of soil; this practice was afterwards abandoned, however, and does not apply to results obtained after 1910. Tests were made also by adding 100 milligrams of dried blood to 100 grams of soil and incubating as above. The soil for the test without dried blood and the soil for the test with dried blood were weighed out at the same time; so that the nitrification of the soil with only its normal supply of organic nitrogen was tested, as well as its nitrifying power when an abundant supply of organic nitrogen was present.

In 1909 the rate of nitrification of soil from the alfalfa plats and from the timothy plats was tested by using ammonium sulfate as described above. The samples were taken to a depth of eight inches on October 6.

In Table 25, which contains the results of these tests, the quantity of nitrates produced in ten days represents the difference between the quantity of nitrates when the samples were taken and the quantity at the end of ten days. The column showing the quantity of nitrates at the end of twenty days has not had the original nitrates subtracted.

TABLE 25. NITRIFICATION IN SOIL UNDER ALFALFA AND UNDER TIMOTHY

Plat	Crop	Soil treatment	Nitrates produced in ten days (parts per million)	Nitrates in soil at end of twenty days (parts per million)
4001 A	Alfalfa	Limed	176	381
4002 A	Timothy	Limed	145	361
4001 C	Alfalfa	Not limed	92	148
4002 C	Timothy	Not limed	77	148

It appears from the results here tabulated that the alfalfa soil, both when limed and when not limed, has a capacity for converting ammonia into nitric acid more quickly than does the timothy soil. This is indicated by the results at the end of ten days. At the end of twenty days, however, the crop factor did not affect the total production of nitrates, at which time there had accumulated about all the nitrates that the nitrifying organisms were capable of producing in the presence of their own products.

The character of the plants grown may therefore affect the rate of nitrification but not the limit of nitrate accumulation in the soil. The former, however, is of greater importance than the latter, as, under field conditions, nitrates are constantly being removed by plant roots or by drainage water and the supply for the growing crops depends on the rate at which nitrates are being formed.

Another test of nitrification in the soil previously under these crops was made on July 6, 1910. The samples for this test were taken from the bare strip on each plat. The results are stated in Table 26.

Nitrate formation is greater in each case in the soil which previously grew alfalfa than in the soil on which timothy had been grown.

In 1911 tests of nitrate formation in the soil under the crops and also in the bare strips on these plats were made. Samples were taken to a depth of eight inches on May 10, May 29, and November 8. In Table 27 are

TABLE 26. NITRATE FORMATION IN SOIL WHICH HAD PREVIOUSLY GROWN ALFALFA AND TIMOTHY, BUT WHICH WAS KEPT FREE FROM VEGETATION DURING THE YEAR IN WHICH TEST WAS MADE

Plat	Soil treatment	Previous crop	Nitrates produced in seven days (parts per million)
4001 A	Limed	Alfalfa	33
4002 A	Limed	Timothy	29
4001 C	Not limed	Alfalfa	26
4002 C	Not limed	Timothy	11

recorded the nitrates produced in fourteen days incubation. These figures represent the differences between the nitrates at the end of the incubation period and the nitrates in the soil as it came from the field.

TABLE 27. NITRATE FORMATION IN SOIL FROM PLATS ON WHICH ALFALFA AND TIMOTHY WERE GROWN AND FROM UNPLANTED SECTIONS OF THESE PLATS

Plat	Crop	Nitrates produced in fourteen days (parts per million)		
		May 10	May 29	November 8
4001 A, 4001 C	Alfalfa	48	59	48
4001 A, 4001 C	None	48	40	16
4002 A, 4002 C	Timothy	50	41	34
4002 A, 4002 C	None	42	33	15

Except in the first test made, the alfalfa soil showed a greater nitrate production than did the timothy soil. In every case the bare soil previously planted to alfalfa nitrified more rapidly than did the bare soil previously planted to timothy. In the test of November 8 the nitrates in the bare soil as it came from the field were so high that the increase during incubation was probably curtailed by the inhibiting action of the products of the nitrifying bacteria.

The natural inference from these results is that in some way the growth of alfalfa renders this soil capable of developing nitrates more rapidly than does the growth of timothy, and that this quality persists in the soil for at least one or two years after the crops are removed.

In addition to the incubation tests of the natural soil, further experiments were conducted by adding to 100 grams of fresh soil 100 milligrams of dried blood and incubating as before. In Table 28 appears a statement of the quantity of nitrates produced in fourteen days under these conditions, and also the excess of nitrates when the soil is incubated with dried blood over those formed by incubation of the natural soil:

TABLE 28. NITRATES PRODUCED BY INCUBATION WITH DRIED BLOOD (PARTS PER MILLION)

Plat	Crop	Nitrates produced in fourteen days		Excess of nitrates with dried blood over those with direct incubation	
		May 10	May 29	May 10	May 29
4001 A, 4001 C	Alfalfa	172	177	124	118
4001 A, 4001 C	None	196	150	148	110
4002 A, 4002 C	Timothy	133	108	83	67
4002 A, 4002 C	None	130	105	88	72

Here again there is shown to be a more active nitrate production in the alfalfa soil than in the timothy soil. The object in incorporating dried blood with the soil was to insure the presence of a large quantity of easily ammonifiable and nitrifiable nitrogen, thus making it possible to measure the activity of the ammonifying and nitrifying organisms independently of the native nitrogenous matter which might be present in larger quantity in the alfalfa than in the timothy soil. The activity of the nitrate-producing bacteria may therefore best be represented by the values obtained by subtracting the nitrates produced by direct incubation from those obtained by incubation with dried blood. The two last columns of Table

28 give these results. In both tests the nitrate-forming bacteria in the alfalfa soil are more active than are those in the timothy soil.

From these data the conclusion may be drawn that the growth of alfalfa on this soil has given rise to greater activity on the part of the nitrate-producing bacteria, or that at least it has not had so pronounced an inhibiting action as the timothy has had.

It is difficult to determine whether the effect of the alfalfa on the soil has been to increase the activity of the nitrifying bacteria as compared with the unplanted soil, or only as compared with the timothy soil. In other words, it may be the case merely that alfalfa depresses nitrification less than does timothy. One reason why it is difficult to ascertain the relative activity of the nitrifying bacteria in the planted and the unplanted soil is because nitrates are higher in the unplanted soil as it comes from the field and the increase during incubation reaches a point at which the products formed may interfere with further action.

The columns in Table 28 in which is stated the excess of nitrates when the soil was incubated with dried blood over those with direct incubation, possibly give some light on this question. It will be noticed that the planted soil produced about the same excess, in the main, as did the unplanted soil. Unfortunately, incubation with dried blood was not performed in the test made later in the season. It seems probable that the character of the organic matter left in the soil by the plants determines to some extent the rate of nitrification in this soil. This is indicated by the

TABLE 29. NITRATES PRODUCED BY INCUBATION BOTH WITH AND WITHOUT DRIED BLOOD IN UNLIMED SOIL (PARTS PER MILLION)

Plat	Crop	Nitrates produced in fourteen days				Excess of nitrates with dried blood over those with direct incubation	
		May 10		May 29			
		Incubation with natural soil	Incubation with dried blood	Incubation with natural soil	Incubation with dried blood	May 10	May 29
4001 C	Alfalfa	66	213	82	227	147	145
4001 C	None	65	206	42	193	141	151
4002 C	Timothy	56	169	47	136	113	89
4002 C	None	49	170	29	112	121	83

fact that, while with incubation of the natural soil the rate of nitrification is more rapid in the planted than in the unplanted soil, yet when nitrification is independent of the native organic matter the rate is practically the same for both the planted and the unplanted soil.

Another question that presents itself is whether the rate of nitrate formation in the unlimed soil would be in the order named. In Table 29 the quantities of nitrates produced during incubation, both with and without dried blood, are stated for the unlimed soil from the alfalfa and the timothy plats. It is seen from this table that the rate of nitrate formation is in the same order as in Table 28.

The nitrate content of alfalfa soil and of timothy soil

Determinations of nitrates in soil growing alfalfa and in soil growing timothy were made from time to time from the summer of 1906, when the seeding was done, to the autumn of 1911. In 1910 and 1911 nitrates were determined also in the unplanted sections of these plats. The nitrate content of the planted sections of the plats during the first year of growth of alfalfa and of timothy, and of both the planted and the unplanted sections during the last two years, are given in Table 30:

TABLE 30. NITRATES IN SOIL UNDER ALFALFA AND UNDER TIMOTHY, AND IN BARE SOIL PREVIOUSLY PLANTED TO THESE CROPS (PARTS PER MILLION)

Crop	1906			1910	1911			
	May 25	July 16	September 6	July 6	May 10	May 29	July 31	November 8
Alfalfa	37	23	11	21	11	10	8	5
None	......	......	......	42	39	65	121	145
Timothy	31	21	8	4	4	6	1	3
None	......	......	......	21	18	32	50	129

It may be seen in Table 30 that the nitrates under timothy are somewhat lower than under alfalfa, but that the difference is not great especially when compared with the nitrates in the unplanted soil. The indications are, therefore, that alfalfa either absorbs nitrates from the soil or depresses nitrate formation to a very great extent. If, however, the nitrates under alfalfa are compared with those in the bare soil previously planted to alfalfa, and then the nitrates under timothy are compared with those in the bare soil previously planted to timothy, it is evident that the nitrates are relatively much lower under alfalfa. As the nitrates in the unplanted soil represent the available nitrates for each crop, it is apparent that alfalfa either has a much greater effect than has timothy in inhibiting nitrate formation or is using more nitrates than is timothy. But it has been shown previously that alfalfa has a stimulating effect on nitrate formation as compared with timothy. It must be concluded, therefore, that, under similar conditions, alfalfa on this soil is absorbing more nitrates than is timothy.

Since there was some doubt in regard to the relation that these two crops may have to the nitrate content of the soil below the surface foot — especially as alfalfa is a much deeper-rooting plant than timothy — the soil was sampled on July 6, 1910, to a depth of four feet and the samples from each foot were analyzed separately. The same number of borings were taken for these analyses as for the others. Both the planted and the unplanted sections of the plats were sampled. The results of the analyses are given in Table 31.

It is seen from this table that the depths below the surface foot contain very small quantities of nitrates under both crops, and even in the unplanted soil the nitrates below the first foot do not change the relations that exist in the surface soil. The samples were taken in midsummer, when the nitrates would have had ample opportunity to leach into the lower soil; they were obtained five years after the crops were planted, during which time the alfalfa roots had penetrated much beyond the upper four feet of soil. It may safely be assumed that the nitrates in the first foot of soil under these crops give an adequate conception of the relative nitrate content of both crops.

Nitrifying activity of soil under crops as shown by inoculation tests

Further experiments were conducted with maize, oats, and bare soil, in which the nitrifying activity of the soil from a number of the plats was maintained at a temperature favorable for nitrification and at an optimum moisture content for a number of days, both when dried blood was added to the soil and when the soil was untreated. The object in adding dried blood was to have in the soil an abundance of easily ammonifiable and nitrifiable organic matter and thus eliminate the errors that might arise

TABLE 31. NITRATES IN SOIL UNDER ALFALFA AND UNDER TIMOTHY, AND IN SOIL THAT HAD PREVIOUSLY GROWN THESE CROPS BUT THAT WAS KEPT FREE FROM VEGETATION. SAMPLES TAKEN JULY 6, 1910

Plat	Depth (feet)	Crop on soil when sampled			Bare soil	
		Crop	Soil treatment	Nitrates (parts per million)	Previous crop	Nitrates (parts per million)
4001 a...	1	Alfalfa.......	Limed.......	14	Alfalfa.......	46
4001 a...	2	Alfalfa.......	Limed.......	1	Alfalfa.......	7
4001 a...	3	Alfalfa.......	Limed.......	1	Alfalfa.......	5
4001 a...	4	Alfalfa.......	Limed.......	1	Alfalfa.......	3
Total...	....	....	....	17	....	61
4002 a...	1	Timothy.....	Limed.......	6	Timothy.....	30
4002 a...	2	Timothy.....	Limed.......	1	Timothy.....	3
4002 a...	3	Timothy.....	Limed.......	1	Timothy.....	3
4002 a...	4	Timothy.....	Limed.......	1	Timothy.....	Trace
Total...	....	....	....	9	....	36
4001 c...	1	Alfalfa.......	Not limed....	28	Alfalfa.......	39
4001 c...	2	Alfalfa.......	Not limed....	3	Alfalfa.......	14
4001 c...	3	Alfalfa.......	Not limed....	2	Alfalfa.......	3
4001 c...	4	Alfalfa.......	Not limed....	Trace	Alfalfa.......	1
Total...	....	....	....	33	....	57
4002 c...	1	Timothy.....	Not limed....	3	Timothy.....	12
4002 c...	2	Timothy.....	Not limed....	Trace	Timothy.....	3
4002 c...	3	Timothy.....	Not limed....	Trace	Timothy.....	Trace
4002 c...	4	Timothy.....	Not limed....	Trace	Timothy.....	Trace
Total...	....	....	....	3	....	15

through differences in the soil in this respect. The nitrate content of the soil as it came from the field was then subtracted from the nitrate content after incubation without dried blood, in order to ascertain the nitrifying activity of the soil. The nitrates formed in the soil treated with dried blood less the nitrates in the field soil possibly represent the efficiency of the nitrate-forming bacteria more nearly than do the nitrates produced either by direct incubation or by incubation with dried blood. In Table

32 these values are given, and also the figures for the nitrates formed by incubation with dried blood less those formed by direct incubation, which probably also represent the efficiency of the nitrate-forming bacteria.

The method used in measuring the nitrate-forming activity of the soil was as follows: The soil was sampled by means of a soil auger to a depth of eight inches. The borings from the plat or section of plat to be tested were mixed, quartered, and placed in an air-tight jar while still moist. When the jar was brought to the laboratory, moisture and nitrates were determined within twelve hours after sampling. Nitrates were determined in 100 grams of moist soil by means of the disulfonic-acid method. Another 100 grams of moist soil was placed in a bottle having a capacity of 250 cubic centimeters, and sufficient water was added to bring the moisture content to 25 per cent of the dry weight of the soil. A loose cotton plug was inserted into the mouth of the bottle, which was then placed in an incubator and kept at a temperature of 30° C. and at a constant moisture content for fourteen days, at the end of which time nitrates were determined. Tests were made also by adding 100 milligrams of dried blood to 100 grams of fresh soil and incubating as above. The samples of soil for tests with dried blood and those for tests without dried blood were weighed out at the same time, so that the conditions of the test were similar except for the addition of the dried blood.

TABLE 32. Nitrate Formation in Planted and Unplanted Soil Incubated with and without Dried Blood, in 1911 (Parts per Million)

Plat	Crop and treatment	Nitrates in natural soil less field nitrates			Nitrates in soil plus dried blood, less field nitrates		Nitrates in soil plus dried blood, less nitrates from incubation with natural soil		
		June 16	July 10	October 9	June 16	July 10	June 16	July 10	August 4
2201, 2209 2401, 2409	Maize, mulched......	32	46	26	110	142	78	96	......
2201, 2209 2401, 2409	None...............	33	37	26	126	125	93	88	......
2203, 2211 2403, 2411	Maize, cultivated.....	40	56	30	119	141	79	85	......
2203, 2211 2403, 2411	None...............	39	45	32	139	127	100	82	......
2207, 2215 2407, 2415	Oats................	46	40	30	105	115	59	75	51
2207, 2215 2407, 2415	None...............	57	37	39	118	111	61	74	50

Considering first the nitrates formed by incubation of the natural, or untreated, soil, it will be seen that on June 16 the maize-planted and the bare soil showed approximately equal nitrate formation. At this time the maize was about eight inches high. On the same date the soil from the oats, which were about a foot high, gave less nitrate formation in the planted than in the unplanted soil. On July 10 both the maize and the oat soil produced more nitrates than did the bare soil. The maize soil was particularly active in this respect. On October 9 nitrate formation was less in the planted than in the unplanted soil. On July 10 the maize had not yet started to tassel and the oats were in a late stage of bloom. On October 9 growth had ceased.

Incubation with dried blood on June 16 shows less nitrate formation in the planted than in the unplanted soil of both crops. The incubations of July 10 show more nitrate formation in the planted soil, for both maize and oats. With an abundant supply of organic nitrogen the nitrate-forming bacteria were very active in the planted soil at this stage of growth, which is an active stage for both these plants. These results are in line with the results obtained by determinations of nitrates in the field; in those tests it was sometimes found that during the active stages of growth the nitrates in the maize soil were higher than those in the corresponding unplanted soil. Nitrates under oats in the field were never so high as in the bare soil. It was concluded from analyses of the field samples that, while the oat plant probably stimulates the formation of nitrates during some stages of its growth, the effect which it exerts in this way is not so great as that produced by the maize plant. The results obtained by these incubation tests show a greater nitrate production in the maize soil than in the oat soil.

There is one essential difference between nitrate formation in the field soil and in the soil brought to the laboratory and placed in the incubator: the latter soil is thoroughly aerated, and some conditions favorable or inimical to nitrate formation may be dispelled.

On October 9 incubation tests were made only with the natural soil. Nitrate production on that date was less in the planted soil. This is again in line with the results obtained from field samples, in which the nitrate content was always very low under a crop during the later stages of growth or after maturing. The incubation tests show a markedly depressing influence for the oat crop and a slight depression for the maize crop. The depressing effect of the oat crop on nitrate formation was shown, by other experiments, to be carried over to the succeeding year, when land previously in maize contained uniformly a higher nitrate content than did land previously in oats.

The depressing influence of the plant is thus shown to be an indirect one, and to remain with the soil for a considerable length of time. Neither influence is completely nullified by aeration, at least by such aeration as is involved in the process of sampling the soil and transferring it to bottles for incubation, although this operation necessitates a very thorough stirring of the soil. It is altogether probable, however, that the depressing influence is decreased by this process. It is much more marked in field tests than in incubation tests; but the former always involve the removal of nitrates by the crop, which introduces an unknown factor. Data will be presented later, showing that freezing dispels in part the depressing effect of the plant on nitrate formation.

Formation of nitrates in soil after freezing and thawing

It has been suggested by the writers that plants which mature on the soil have a depressing effect on nitrate formation in that soil for some time afterwards. If this is the case, it seems probable that the inhibiting effect of the crop may be removed in greater or less degree by subjecting the soil to certain treatments. The effect of aeration on formation of nitrates is well known. To what extent freezing may affect the nitrifying property of a soil is not so well understood, as few experiments on that subject have been published.

Recently Brown and Smith (1912) reported that in ammonification tests in peptone solutions and in soil to which infusions of field soil were added at intervals from October 17 to March 1, the ammonifying power was greater after the soil had become frozen than before. Similar tests of the nitrifying power of the field soil did not indicate an increase in the nitrifying power of the frozen soil, but these investigators did not consider their results in this experiment conclusive. Four pots of soil that had been in the greenhouse for a year and had raised a crop of wheat and a crop of millet during that time were used in the experiment. Pots 127 and 128 contained the same kind of soil and had received the same treatment, and pots 343 and 344 corresponded with each other in these respects. The two first-mentioned pots contained Volusia silt loam and the other two contained Dunkirk clay loam.

On December 28, 1911, pots 127 and 343 were placed in a building in which the temperature corresponded closely with that of the outside air but the moisture content of the soil could be controlled. Pots 128 and 344 remained in the greenhouse. All the pots were kept bare of vegetation and were maintained at a moisture content of about 25 per cent of the dry weight of the soil. On February 28, 1912, pots 127 and 343 were returned to the greenhouse. During most of the time that they were in the outside building they were frozen, and two or three times they thawed. They stood in the greenhouse with pots 128 and 344 until March 4, when all were brought to the laboratory and nitrates were determined. The results are shown in Table 33:

TABLE 33. NITRATES IN SOILS SUBJECTED TO FREEZING AND IN SIMILAR SOILS REMAINING UNFROZEN

Pot	Kind of soil	Treatment	Nitrates (parts per million)
127	Volusia silt loam	Frozen	155
128	Volusia silt loam	Unfrozen	112
343	Dunkirk clay loam	Frozen	92
344	Dunkirk clay loam	Unfrozen	70

It would appear from this table that freezing produces a condition of soil favorable for nitrate formation. The well-known action of frost in producing a granular condition of a clay soil may have operated to some extent to bring about this condition. To what extent the more active production of nitrates is due to aeration and to what extent it is due to cold, it is impossible to say; but the obvious effect is to overcome the depressing influence of the crop previously grown and to permit the resumption of nitrate formation required for the growth of the crop that follows.

The depressing influence of grass on nitrate formation a possible factor in orchard management

The experiments by Dehérain and by Leather, already cited, indicate that all the plants tested except maize depressed the formation of nitrates to such an extent that the nitrogen in the crop plus that in the drainage water was less than the nitrogen in the drainage water from an unplanted lysimeter containing a similar soil. These results are in part confirmed by experiments conducted by the writers with somewhat similar apparatus. The writers have had only two years in which to use this set of tanks, and hence the data are probably not entirely reliable.

Experiments were conducted in large concrete tanks filled with soil. The tanks are four feet two inches square and four feet deep and each tank holds about three and one half tons of soil. The soil used was from the experiment field in which most of the other experiments with nitrate formation were conducted. The tank soil receives the natural rainfall and no other water supply. The drainage water is all collected and measured and samples are taken for analysis. In 1910 four tanks were planted to maize, two were planted to oats, and three remained unplanted. In 1911 four tanks were in oats, two were in grass, and two were bare of vegetation. The unplanted tanks were cultivated in 1910, and in 1911 they were scraped in order to remove weeds without stirring.

In Table 34 are given the quantities of nitrogen in the drainage water, and in the crop, including the estimated quantities in the roots, for the periods already stated.

The results do not agree in every respect with those obtained by Leather and by Dehérain. Both the tanks planted with maize and those planted with oats contained a larger quantity of nitrogen in the crop and the drainage water combined than the unplanted tanks contained in the drainage water. The grass-planted tanks, like those of the experimenters just cited, contained notably less. In fact, less than two thirds as much nitrogen was found in the combined crops and drainage from the grass tanks as was found in drainage from the bare tanks.

The quantity of nitrogen removed by the crop and the drainage water combined is in the same order with respect to the kind of plant grown as is the nitrate content of the soil, as shown by the plat experiments. Thus, the nitrogen removed from the maize plats was greatest, that from the oats plats was next, and that from the grass plats was least; and the nitrate content of the maize soil is higher during the growing season than is that of oats, and the latter soil has uniformly a higher nitrate content than has grass land. These results are shown graphically in Diagram XXV.

TABLE 34. REMOVAL OF NITROGEN FROM PLANTED AND UNPLANTED SOIL TANKS

(May 23, 1910, to May 1, 1911)

Tanks	Crop	Nitrogen (pounds per acre) in			
		Drainage	Tops	Roots* (estimated)	Tops, roots, and drainage
2, 4, 8	None	120	....	....	120
3, 5, 7, 9	Maize	11	140	47	198
6, 10	Oats	12	90	30	132

(May 1, 1911, to May 1, 1912)

Tanks	Crop	Nitrogen (pounds per acre) in			
		Drainage	Tops	Roots* (estimated)	Tops, roots, and drainage
4, 8	None	68	....	....	68
3, 5, 7, 9	Oats	5	52	17	74
6, 10	Grass	1	30	10	41

* Nitrogen in roots estimated as one third that in tops.

If plants have a stimulating influence on nitrate formation during the earlier growing stages and a depressing influence later, the nitrogen in the drainage water plus the nitrogen in the crop will be greater or less than the nitrogen in the drainage water from the unplanted soil, in proportion to the relative activities of the two influences and also to the extent to which the plant in question absorbs nitrogen in forms other than as nitrates.

In Dehérain's experiments maize gave about as much nitrogen in the crop and the drainage water as was contained in the drainage water of the

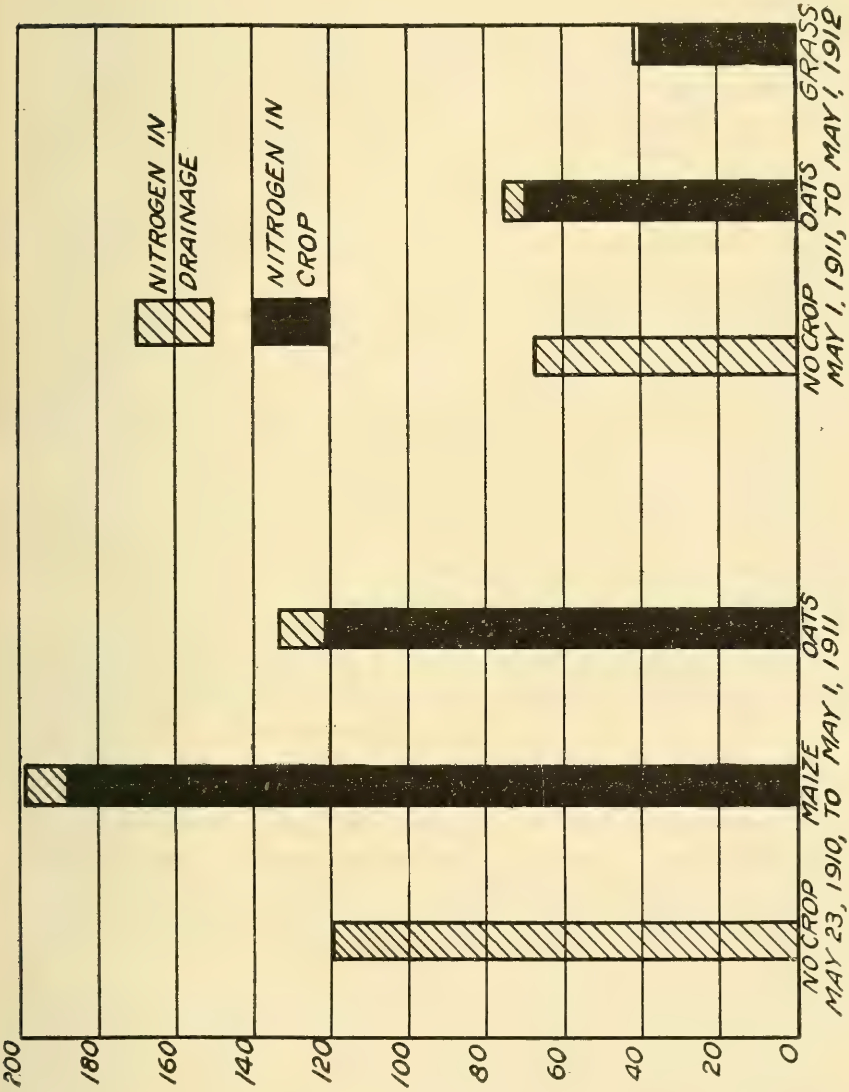


DIAGRAM XXV.— *Number of pounds of nitrogen removed per acre from planted and unplanted soil tanks* uncropped soil; in the writers' experiments maize soil afforded a much larger quantity of nitrogen in the crop and drainage. Oats also, in the writers' experiments, accumulated more nitrogen in the crop and drainage water than was contained in the water from the bare soil. Grass, however, gave results of a nature similar to those obtained by Dehérain, but in less degree.

It is evident that in the case of grass the depressing influence on nitrate formation is greater than the stimulating influence.

The discrepancy between the writers' results and those obtained by Dehérain and by Leather may be accounted for in any one or more of the following ways:

1. On the soil used by the writers the plants may have absorbed a larger proportion of their nitrogen in forms other than as nitrates.

2. Possibly the very heavy clay soil used in the writers' experiments retained a larger proportion of nitrates, and this would have affected the bare soil more than it would the planted soil as the quantity of nitrates in the former was much greater than that in the latter.

3. The lower layers of the soil may have been the scene of a denitrifying process, which would probably have decreased the nitrogen removed from the bare soil more than that from the planted soil because there were more nitrates in the bare soil to be denitrified.

4. The stimulating influence of the higher plants on nitrate formation may have been greater in comparison with the depressing influence in the soil used by the writers than in the soils used by Dehérain and Leather.

The evidence afforded by the experiments of Dehérain and Leather indicates that wheat, oats, potatoes, grass, and beets have a greater depressing influence than they have stimulating influence on nitrate formation, and that the stimulating and the depressing influence in maize practically balance each other. The high nitrate content in maize soil on the writers' experiment field in the spring following the growth of the maize crop, as well as the results from the soil tanks, indicates that on this soil the stimulating influence of maize is greater than its depressing influence.

It appears from these considerations that the three kinds of plants grown in the tanks at this station exerted a greater stimulating influence on nitrate formation than did the same plants at Grignon, and that the extent to which higher plants affect nitrate formation favorably or unfavorably varies in different soils. The soil used by the writers is evidently very favorable to nitrate formation.

That grass exercises a strongly depressing action on nitrate formation is indicated not only by the three sets of tank experiments cited above, but also by the determinations of nitrates under timothy sod previously presented in this paper. This characteristic of the grasses almost completely prevents a loss of nitrogen in the soil drainage and thus tends to conserve nitrogen, which is doubtless one of the factors in the accumulation of nitrogen in grassland.

The same properties of grass with respect to nitrate formation may be a factor in the effect of grass in orchards.

Stewart (1911) has lately called attention to the need of nitrogen in orchard fertilization. Experiments indicate that under some conditions the use of nitrate fertilizer has been very effective in increasing the growth and yield of apple trees. In sod-covered orchard soil the supply of nitrates must be kept very meager by the grass, and if the other forms of nitrogen in the soil are not such as to afford a suitable supply the trees must suffer from lack of nitrogen. Where trees apparently thrive on sod, a part of their nitrogen supply may come from some nitrogenous compounds other than nitrates.

The plants used as cover-crops doubtless exercise an influence in this respect. The practice of fallowing an orchard until July and then planting a cover-crop is in accordance with the most economical management of the soil nitrogen. In this way the formation of nitrates is favored during the early part of the summer, and is depressed when the supply is no longer needed by the trees and would otherwise be leached from the soil. This is, of course, assuming that nitrates are utilized by apple trees as a source of nitrogen.

The influence of a preceding crop on nitrate formation in soil

While the practices of crop rotation have been fairly well worked out, there yet remains much to learn regarding the underlying principles. Most of the effort in experimentation with soils has been made with a view to ascertain the effect of certain soil conditions on plant growth or on the solubility of the mineral nutrients in various solvents. The study of the principles of crop rotation necessitates the investigation of the effect of plant growth on the soil. The fact that a certain plant grows better when preceded by one species of plant than when preceded by another is a self-evident indication that plants of different species exert different influences on the soil.

The writers have already suggested that certain higher plants have a distinct influence on the process of nitrification in the soil, and that this influence varies in intensity with plants of different families and at different stages in the growth of any one kind of plant. It was found, for instance, that in a soil on which maize was growing the nitrates were sometimes higher at certain periods than in a similar soil on which no plants grew. Timothy (*Phleum pratense*), on the other hand, maintained uniformly a very low nitrate content in the soil throughout the growing period.

The question then arose whether the higher plants exert any influence on the activities of the nitrifying bacteria after the plants are removed. In order to test this it was decided to continue the nitrate determinations for a second year, in soil that had been planted to several kinds of crops.

Plats that had been used in 1910 for studying the effect of plants on the nitrate content of soils were left bare in 1911 until July 1. In order to keep down weeds the plats were disk-harrowed from time to time. Samples of soil were taken from the sections of the plats that were planted and those that were bare in 1910, for the purpose of ascertaining the effect on the nitrate content of the soil of the different crops grown in the previous year. The borings were made to a depth of eight inches below the surface, and from eight to sixteen inches below the surface. In Table 35 are presented the results averaged for the entire season for the four plats planted to the same crop, also the averages of the unplanted sections of the same plats during the growing season of 1910. The table consequently shows the nitrate content of the soil in the year during which the test crops were grown.

TABLE 35. NITRATES IN PLANTED AND UNPLANTED SECTIONS OF PLATS. AVERAGE FOR THE GROWING SEASON, 1910

Plat	Crop	Nitrates (parts per million)	On basis of nitrates in unplanted soil taken as 100	Nitrogen in crop (pounds per acre)
3612, 3613, 3622, 3623	Maize	167	123	3
	None	136	100	....
3614, 3615, 3624, 3625	Potatoes	104	96	43
	None	108	100	....
3616, 3617, 3626, 3627	Oats	90	71	29
	None	126	100	....

The nitrates were highest under the maize, next highest under the potatoes, and lowest under the oats. Similar results had been obtained in previous years.

The last column of Table 35 shows the quantity of nitrogen removed per acre by each crop. The maize crop was a very poor one, which accounts for the small quantity of nitrogen contained in that crop. It is doubtless true that the small maize crop is partially responsible for the high nitrate content of the maize soil; however, this high nitrate content has been found in maize soil in previous years when the crop was good. Again, the persistently higher nitrate content of the maize soil as compared with the unplanted soil can be explained only on the assumption that nitrate formation is much more active where the maize is growing, for certainly more nitrates are being removed from the planted soil.

Nitrates are higher in the potato soil than in the oat soil, although the former crop removed nearly fifty per cent more nitrogen than did the latter. The nitrate content of a planted soil is not determined by the total quantity of nitrogen removed by the crop, but by a number of factors, of which one appears to be a direct influence which each species of plant exerts on the activity of the nitrifying organisms.

In the year following that in which the results given above were obtained, these plats were kept free from vegetation until July 1 and nitrates were determined from time to time. In Table 36 is given a statement of the nitrates in the previously planted and in the unplanted sections of these plats, on each of the dates when the soils were analyzed:

TABLE 36. NITRATES IN SOIL PLANTED IN THE PREVIOUS YEAR AND UNPLANTED IN 1911

Plat	Crop	May 1		June 5		June 28	
		Surface eight inches	Second eight inches	Surface eight inches	Second eight inches	Surface eight inches	Second eight inches
3612, 3613, 3622, 3623	Maize....	51	53	78	45	40	34
	None....	48	52	68	44	40	30
3614, 3615, 3624, 3625	Potatoes..	31	26	50	26	29	24
	None....	43	43	80	43	35	29
3616, 3617, 3626, 3627	Oats.....	23	21	41	33	22	22
	None....	32	40	68	35	34	32

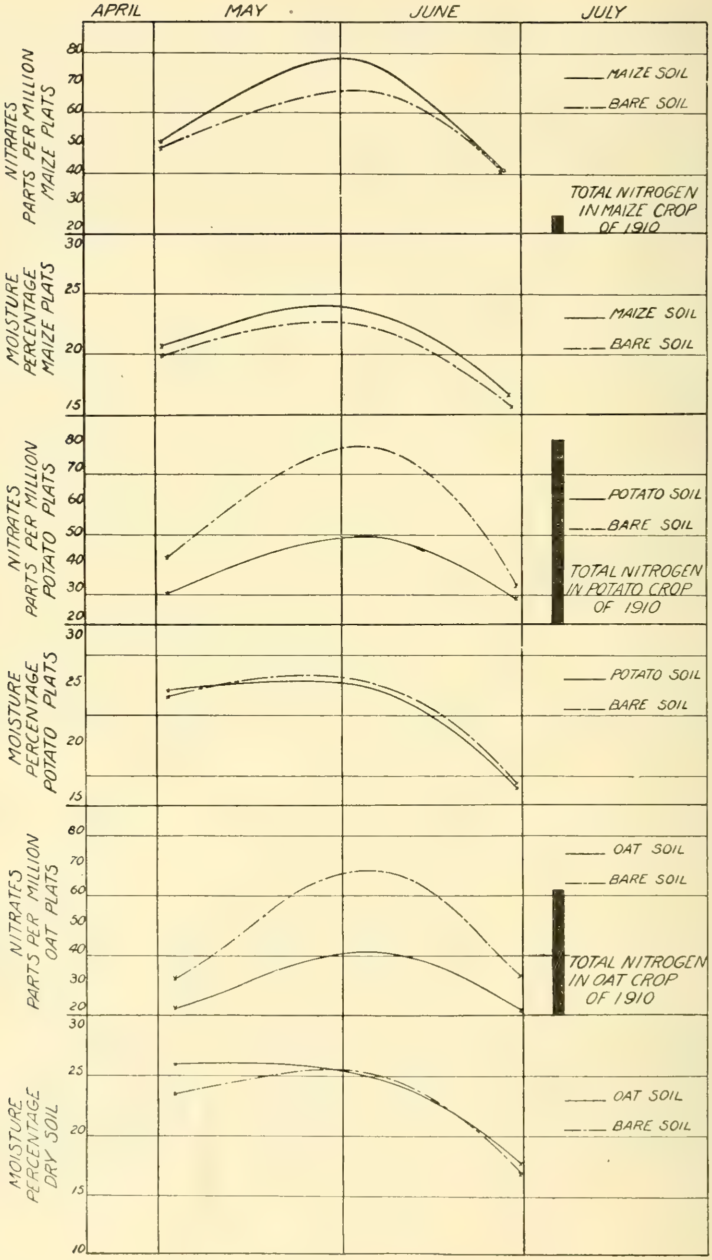


DIAGRAM XXVI.— *Nitrates and moisture in surface eight inches of soil plats kept bare of vegetation in 1911, which had been planted in 1910 to maize, potatoes, and oats with bare sections on each plat*

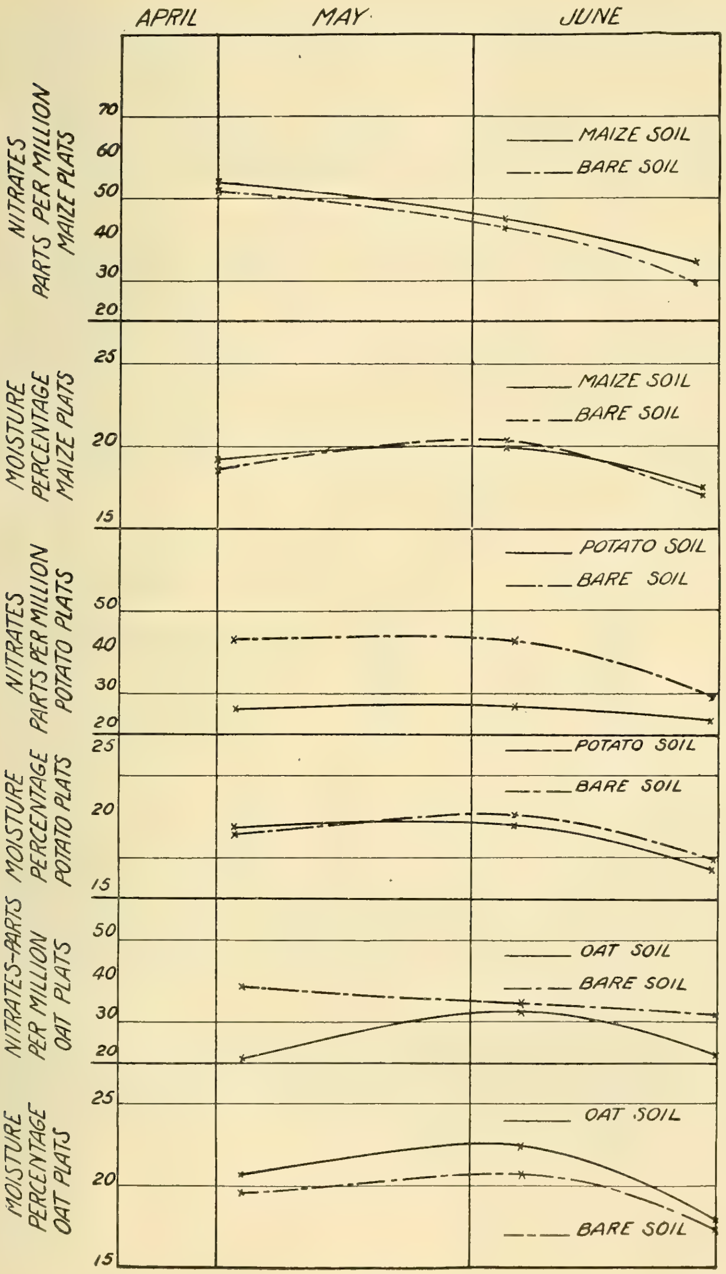


DIAGRAM XXVII.— *Nitrates and moisture in second eight inches of soil plats kept bare of vegetation in 1911, which had been planted in 1910 to maize, potatoes, and oats with bare sections on each plat*

An examination of this table shows that, except in the case of maize, the nitrates are lower in the soil on which plants grew during the previous year than in the soil kept unplanted during that time. It will be noticed also that the nitrates on the unplanted soil are in the same order as they were when the plats were planted. Thus, nitrates are highest in the soil previously planted to maize, next in that planted to potatoes, and lowest in that planted to oats. This is brought out also in diagrams XXVI and XXVII.

In Table 37 is given a statement of the nitrates in the planted sections of the plats in terms of the nitrates in the unplanted sections taken as 100. In order to obtain these figures the nitrates in both planted ends of each of the four plats planted to the same crop are averaged, and this average is divided by the average for the nitrates on the unplanted sections of the corresponding plats. This furnishes the most convenient means of comparison, as it removes more effectively than any other method the local variations in the nitrate content of the soil.

TABLE 37. RATIO OF NITRATES IN SOIL OF PLATS PREVIOUSLY PLANTED TO NITRATES IN UNPLANTED SOIL

Plat	Crop	Surface eight inches			Second eight inches		
		May 1	June 5	June 28	May 1	June 5	June 28
3612, 3613, 3622, 3623	Maize.....	106	115	100	102	102	113
3614, 3615, 3624, 3625	Potatoes..	72	62	83	60	60	83
3616, 3617, 3626, 3627	Oats.....	72	60	65	52	94	69

There is a distinct and characteristic difference in the nitrate content of the soil previously bearing these different plants. While the experiment covers only one season, the differences would seem to be too large and too well-defined to be accidental.

On July 1 the plats used in this experiment were drilled to millet. The entire plats were planted, including the middle section of each plat which during the preceding year had remained unplanted. The object in planting millet was to ascertain how the growth of a crop on these plats would correspond with the nitrate content.

On September 8 careful notes were taken of the growth on each plat, and a comparison was made not only between different plats but also between the growth of millet on the previously planted sections and on the unplanted section of each plat.

In each plat the growth of millet was markedly better on the sections that had been in crop during the previous year than on the unplanted section. This applied to every crop in the experiment. A comparison of the growth on the planted sections of the plats is given in Table 38, in which the growth of millet on the plats planted to oats is taken as 100 and the growth on the other plats is stated on this basis:

TABLE 38. GROWTH OF MILLET ON PLATS PLANTED TO DIFFERENT CROPS IN THE PRECEDING YEAR

	Soil treatment	Plat	Growth of millet (percentage)	Plat	Growth of millet (percentage)	Average growth of millet (percentage)
Maize	Not limed	3612	37	3622	62	49
	Limed	3613	47	3623	71	59
Potatoes	Not limed	3614	60	3624	81	70
	Limed	3615	65	3625	88	76
Oats	Not limed	3616	100	3626	100	100
	Limed	3617	100	3627	100	100

It will be seen that the luxuriance of the growth of millet on these plats was inversely proportional to the relative concentration of nitrates in the soil. For instance, the millet made the best growth on the plats planted to oats, and these plats had the lowest nitrate content. The poorest growth of millet was on the maize plats, and these had the highest concentration of nitrates.

In Table 39 the relative nitrate content in the surface eight inches of the several plats on June 28, which was only a few days before the millet was planted, is compared with the percentage growth of millet on the same plats:

TABLE 39. COMPARISON OF NITRATES IN SOIL PLANTED TO CERTAIN CROPS WITH GROWTH OF MILLET ON THE SAME PLATS

Crop in 1910	Relative nitrate content	Relative growth of millet
Oats	55	100
Potatoes	70	73
Maize	100	54

It would seem from these results that in this soil the conditions favoring the growth of millet were unfavorable to the formation of nitrates. That this is not true of all conditions is evident from the fact that the millet grew better on the limed than on the unlimed soil, as shown in Table 38; while the nitrates also are higher in the limed plats, as may be observed in Table 40, which follows:

TABLE 40. NITRATES IN UNPLANTED SECTIONS OF PLATS LIMED AND NOT LIMED

Plat	Soil treatment	Nitrates (parts per million)			
		May 4	June 5	June 28	Average
3612, 3622	Not limed	40	61	42	48
3613, 3623	Limed	55	75	39	56
3614, 3624	Not limed	40	77	34	50
3615, 3625	Limed	45	82	36	54
3616, 3626	Not limed	28	52	36	39
3617, 3627	Limed	36	85	40	54

It is evident that, although the previous crop influences greatly the nitrate content of this soil, the growth of millet is not increased thereby. Nitrates are not the limiting factor in the growth of millet on this soil.

Lime increases the growth of millet and also the formation of nitrates, but apparently its beneficial action on the growth of millet must be due to some cause other than its influence on nitrate formation.

The purpose of this experiment is to ascertain the influence of certain kinds of plants on the formation of nitrates in the soil after the crops have been removed. The relation of the nitrate formation to the growth of millet on this soil is a separate problem, probably peculiar to this particular soil but possibly worth recording. The interesting condition exists that, of the crops used in the experiment, each had a certain and distinct influence on nitrate formation following the removal of the crops and on the growth of millet also following their removal, but a high nitrate content was not necessarily accompanied by a large crop of millet. It is not to be expected, however, that this lack of correlation will obtain in all soils, since under some circumstances the presence of an abundance of nitrates will throw the influence toward a better growth of the succeeding crop. There is thus presented a problem in crop rotation. If the influence of a certain kind of plant on the formation of nitrates after its removal holds for soils other than the soil used in this experiment, something has been learned. That other influences obtain, and, under some circumstances, influences more potent than nitrate formation, is also indicated by this experiment.

Is there a mutual stimulation of plant growth through root influence?

The stimulating influence of plant growth at certain stages on nitrate formation in soils suggested a possible benefit to higher plants from other and younger plants growing with them. It was decided to start a number of plants of different kinds in the greenhouse, placing them far enough apart to prevent interference, at least while they were young, and after they had attained a reasonable size to sow between them seed of a different kind of plant in order to ascertain whether the plants that were first started received any benefit from the growth of the plants seeded later. If the later-started plants stimulated nitrate formation at a time when the earlier-started plants were beginning to depress it, the latter might receive some benefit from the nitrates formed.

In order to test this question, an ordinary greenhouse bench was divided into compartments eighteen inches square and six inches deep. These were filled with soil of good quality, prepared by rotting timothy sod which had been produced on Dunkirk clay-loam soil. About twenty-five or thirty of the earlier-started plants were grown at equal distances apart in each square. These will be designated as the " primary " plants or crop. Twice as many plants were started later, being arranged one on each side of the primary plants. These will be termed the "secondary" plants or crop. In some cases the secondary plants were planted at the same time as the primary.

For the purpose of checking the experiments in which soil was used for growing plants, a series of tests were made with crushed quartz containing about thirty per cent of its weight of nutrient solution. This solution had a density of 4500 parts per million.

Glazed earthenware pots of a capacity of one half gallon were filled with the quartz and the nutrient solution, and four to twelve plants, depending on the size, were grown as a primary crop in each pot. The secondary crop consisted of twice as many plants. The moisture content was maintained by weighing the pots two or three times a week and adding distilled water in sufficient quantity to make up the loss. Nutrient solution was added two or three times in the life of the plants. It was intended to have an abundant supply of plant nutrients, especially nitrogen in the form of nitrate. The object in using the nutrient solution was to furnish a medium in which the secondary crop could be of no assistance as a means of supplying nitrate nitrogen.

In both the soil and the crushed quartz, the squares and the pots planted with the primary and the secondary crops were in each case accompanied by similar receptacles planted with the primary crop alone and containing exactly the same number of plants as were present as a primary crop in the vessels containing the primary and the secondary, or what will be termed the mixed, crops. The yields of the plants growing alone may thus be compared with the yields of the primary plants in the mixed crops, as a measure of the effect of the secondary crop on the primary crop.

The plants growing in the mixtures were always at a disadvantage because there were always three times as many plants in the mixed crop as in the single one. In order to offset this it was intended to furnish an abundant supply of nutriment, but in the greenhouse soil this was probably not fully accomplished. In the nutrient solution some of the mixed crops possibly lacked nutriment.

The tests in the greenhouse soil in which the primary and the secondary plants were started at the same time did not give very satisfactory results, as the secondary crops grew so large that they perceptibly dwarfed the primary crops except in a few cases. This, however, was anticipated, and such mixtures were used only as checks. In the quartz cultures the primary and the secondary crops were all planted at the same time, in spite of which the secondary crops did not suffer greatly from the competition.

In Table 41 are given the yields of the primary crops in the mixtures as compared with the yields of the same crops in the single cultures when the latter are taken as 100. In this comparison the same number of plants is taken in both the single and the mixed cultures. In some cases the secondary crop grew fairly well and in other cases the growth was slight. The relative yields of the primary crop and the secondary crop in the same squares are stated in the table. The yields are based on weights of the water-free material.

TABLE 41. RELATIVE YIELDS OF PRIMARY CROPS GROWN ALONE AND OF PRIMARY CROPS IN MIXTURES ON GREENHOUSE SOIL

Primary crop	Secondary crop	Yield of primary crop grown in mixture when primary crop alone is taken as 100	Yield of secondary crop when primary crop in mixture is taken as 100	Period of growth of primary crop (days)	Period between planting primary and secondary crops (days)
Dandelion	Timothy	102	20	166	91
Timothy	Mustard	127	13	128	67
Wheat	Mustard	93	0	146	72
Oats	Mustard	132	1	142	72
Oats	Mustard	98	1	87	46
Oats	Maize	133	1	107	72
Oats	Maize	107	1	93	58
Wheat	Maize	97	1	93	61
Millet	Maize	98	17	63	44
Millet	Maize	88	8	84	65
Spring wheat	Winter oats	69	2	151	71
Spring oats	Winter oats	92	2	151	70
Peas	Lettuce	100	40	67	37
Timothy	Redtop	99	15	130	29
Wheat	Rye	112	1	84	49

It is seen from this table that in some instances the yield of the primary crop in the mixture was as large as, or larger than, when grown alone. As this occurs in nearly half of the cases, it appears at first glance that the larger yields are accidental and not due to any influence exerted by the secondary crop. Taken by themselves these results could not be said to indicate anything, although the fact that nearly half of the tests gave larger yields of the primary crops when grown in mixtures than when grown alone is perhaps deserving of attention, especially since in the squares containing mixtures seventy-five plants were grown in six inches of soil eighteen inches square, and consequently there must have been more competition for food in the mixtures than in the pure cultures.

The cultures in ground quartz emphasize this more strongly, as may be seen in Table 42:

TABLE 42. RELATIVE YIELDS OF PRIMARY CROPS GROWN ALONE AND OF PRIMARY CROPS IN MIXTURES IN NUTRIENT SOLUTION IN CRUSHED QUARTZ

Primary crop	Secondary crop	Yield of primary crop grown in mixture when primary crop alone is taken as 100	Yield of secondary crop when primary crop in mixture is taken as 100	Period of growth of plants (days)
Wheat	Mustard	132	34	87
Wheat	Mustard	126	20	87
Wheat	Mustard	106	89	51
Wheat	Mustard	108	100	51
Oats	Mustard	133	19	87
Oats	Mustard	96	73	48
Oats	Mustard	98	99	48
Peas	Lettuce	87	15	83
Peas	Lettuce	76	8	83
Peas	Lettuce	86	8	54
Peas	Lettuce	91	3	54
Timothy	Blue grass	95	50	94
Timothy	Blue grass	78	72	94
Timothy	Blue grass	84	19	67
Timothy	Blue grass	73	26	67
Timothy	Red clover	152	38	94
Timothy	Red clover	116	44	94
Timothy	Red clover	76	180	67
Timothy	Red clover	81	146	67
Red clover	Timothy	123	26	94
Red clover	Timothy	109	44	94
Timothy	Daisy	107	36	93
Timothy	Daisy	124	47	93
Wheat	Red clover	95	20	71
Wheat	Red clover	105	27	71
Wheat	Red clover	96	4	60
Wheat	Red clover	109	5	60
Wheat	Timothy	100	5	59
Wheat	Timothy	111	7	59
Oats	Timothy	102	6	59
Oats	Timothy	75	11	59

In these tests the primary and the secondary crops were planted at the same time. The growth was more rapid than in the soil. Again in about half of the tests the primary crop gave larger yields when grown in the mixture than when grown alone. As any benefit to the primary crop by a stimulation of nitrate formation through the growth of the secondary crop is precluded by the fact that the entire supply of nitrogen was in the form of nitrate, the evidence is opposed to the supposition that any increased growth of the primary crop in the mixtures grown in soil was due to such a process.

An examination of the data recorded reveals two conditions that influence the growth of the primary crop in mixtures. These are the length of the period of growth and the relative growth of the secondary crop. The benefit to the primary crop from a longer period of growth may be due either to a slower growth or to harvesting at a later period of development of the plant. It will be noticed that for any given mixture a very large growth of the secondary crop is usually accompanied by a relatively smaller growth of the primary crop. This holds true only when the yield of the secondary crop is very large, and is presumably due to the competition of the plants for food and water.

It was decided to repeat these tests in the field, where there would be a large body of soil from which the plants could obtain nourishment and where the conditions would be more nearly normal than in the greenhouse. The plants were grown in squares six feet each way and usually twenty-five primary plants were placed in each square. Whether grown alone or in a mixture, the number of primary and secondary plants in each square was the same. The plants were not watered artificially, and as the summer was a very dry one they suffered from lack of moisture. Each combination of plants was repeated on four squares. Two of these were harvested at or near the flowering stage and the other two were allowed to ripen fully. In Table 43 the yields are given for only the squares that were harvested before the plants were mature, as in almost all cases the mature plants yielded more in single cultures than in combination. This may have been due to the severe drought or it may be the natural result under any conditions. At least it is what would have been expected. The relative yields are based on the oven-dry weights of the plants. In choosing the plants for this experiment, combinations were used which previous experience or observation indicated would give larger yields of the primary plants when grown in combination than when grown alone. The field soil used was Dunkirk clay loam.

TABLE 43. RELATIVE YIELDS OF PRIMARY CROPS GROWN ALONE AND OF PRIMARY CROPS GROWN IN MIXTURES ON FIELD SOIL

Primary crop	Secondary crop	Yield of primary crop grown in mixture when primary crop alone is taken as 100	Yield of secondary crop when primary crop in mixture is taken as 100	Period of growth of primary crop (days)	Period between planting primary and secondary crops (days)
Barley	Buckwheat	166	37	67	30
Barley	Buckwheat	126	79	70	30
Oats	Buckwheat	132	35	70	31
Oats	Buckwheat	106	55	73	31
Oats	Mustard	115	37	70	27
Oats	Mustard	102	99	73	25
Barley	Mustard	116	38	67	24
Barley	Mustard	105	39	70	24
Peas	Lettuce	40	172	35	*—35
Peas	Lettuce	58	169	38	—35
Oats	Red clover	104	175	40	—28
Oats	Red clover	122	143	43	—28
Oats	Maize	107	25	68	38
Barley	Maize	94	50	70	37
Barley	Maize	83	34	70	37

* The minus sign indicates that the secondary crop was planted before the primary crop.

In the field soil a major proportion of the tests resulted in a larger yield of the primary crop in the mixtures than when grown alone. As in the previous tests, a particularly heavy yield of a secondary crop was accompanied by a relatively lighter yield of the corresponding primary crop. This is very noticeable in the combinations of barley and buckwheat, oats and buckwheat, and oats and mustard. It is not possible to trace any relation between the influence of the secondary crop and the length of the growing period or the length of time between planting, as the duplicates do not vary much in these respects.

Certain plants that were used as secondary crops were particularly successful in promoting a good growth of the primary crops. These were mustard, buckwheat, maize, and red clover. In the experiments in nitrate formation, maize and legumes were found to have a favorable influence on nitrate formation. The other two crops did not enter into these experiments.

The much more pronounced influence of the secondary crops on the growth of the primary crops in the field soil than in the nutrient solution in crushed quartz leaves some question as to whether the influence of the secondary crops on nitrate formation was a factor in the growth of the primary crops. The result of the experiments with nutrient solution opens the question as to whether there is any other way in which such an influence could be exerted, if indeed the secondary crop really does affect favorably the growth of the primary crop.

It is true that there are certain combinations of plants in which the primary crop made a better growth than it did in other combinations; but in most such cases the primary crop gave a larger yield in the combination than it did alone, thus indicating a favorable influence on the part of the secondary crop and not merely an innocuous one.

From other experiments results have been obtained that indicate a stimulating influence of certain kinds of plants on the growth of other kinds. Dandeno (1909) grew oats, barley, buckwheat, wheat, and flax in soil contained in greenhouse pots, both with and without underground shoots of Canada thistle, and repeated the experiment with a young elm tree. All the crops except buckwheat grew better with the roots of Canada thistle than alone. All grew less with the elm tree. The stimulating effect of the thistle was most pronounced twenty-two days after planting. Dandeno suggested as a cause the excretion of substances that might stimulate the growth of the other plant or release plant food not otherwise available, or, on the other hand, might injure the other plant or tie up soluble nutrient material.

It is reported by the Norsk Landmandsblad (1903) that in Jutland spruce trees make a good growth on waste areas where mountain pines grow, when their roots are in contact, but otherwise not. If the pine is cut down while the spruce is still young the latter will die or will make a sickly growth; if, on the other hand, it is not cut until after several years, the spruce not only will survive but appears to grow faster than would have been the case if the pine had been left standing. The Norwegian experimenters account for this by supposing that pines utilize atmospheric nitrogen and furnish spruces with a supply of nitrogen.

Through what process the stimulation that one plant often seems to exert on another is accomplished, still remains to be ascertained. This apparently resembles, in some ways, the stimulus that certain plants exert on nitrate formation. Thus the stimulus is exerted more strongly during the earlier part than during the later part of the life of the plant. This is indicated by the field experiments already described, in which nearly all the primary crops harvested at bloom gave a larger yield in combination than alone, while similar mixtures that were allowed to mature gave opposite results. As a considerable part of the nutrients absorbed by plants had been taken up when the time of blossoming came, the competition of the secondary crop during the later growth could not have been very severe. It was also noticed that in quartz cultures the secondary crop remained almost stationary during the later growth of the primary crop and made an excellent growth after the latter had ceased growth entirely. Like the effect of the plant on nitrate formation, there appears to be a depressing effect during the later stages of growth. Another point of resemblance is that the influence of certain kinds of plants is more marked than that of other kinds, and in this respect maize and red clover were found to be especially active in stimulating both processes.

The natural growth of plants in associations, in which one plant follows another in succession through a considerable part of the season, may be to some extent a utilization of this apparent property of the plant to stimulate the growth of other plants.

Summary

The nitrate content of soil under timothy, maize, potatoes, oats, millet, and soy beans was different for each crop when on the same soil. There was characteristic relationship between the crop and the nitrate content of the soil at different stages of growth. During the most active growing period of the maize crop, nitrates were frequently higher under maize than in cultivated soil bearing no crop. Under a mixture of maize and millet, nitrates at this period were higher than under millet alone, although the crop yields were about the same on both plats.

These phenomena may be accounted for on the assumption that nitrate formation is stimulated by some processes connected with the active growth and absorbing functions of some higher plants, particularly of maize, although there are indications that the maize plant obtains a large part of its nitrogen in some form other than as nitrates; the combination of these conditions may account for the very high nitrate content of the soil under maize.

Under both maize and oats the nitrate content was higher during the period when the crop was making its greatest draft on the soil nitrogen than in the later stages of growth, in spite of the fact that the nitrates in the uncropped soil were increasing while those in the cropped soil were disappearing. Nitrates under these crops and under millet failed to increase late in the season, when nitrogen absorption had practically ceased, although uncropped soil showed a very large increase in nitrates at that time.

This, in conjunction with facts before mentioned, indicates a further influence of the crop on the process of nitrate formation, and may be accounted for on the supposition that during their later period of growth the plants exert in some manner a depressing influence on nitrate formation.

Aside from the influence of cultivation, the source of the great differences in the nitrates under the crops mentioned may be sought in the inherent differences between plants of different species in their stimulating or inhibiting influence on the production of nitrates, as well as in their relative rates, amounts, and forms of nitrogen absorption.

Changes in the moisture content or in the temperature of the soil after early summer had no important effect on the nitrate content of the soil under plants. On the uncropped soil an increase in moisture content was sometimes accompanied by an increase in nitrates and sometimes by a decrease.

Determinations of the rate of nitrification of soil from plats planted to alfalfa and timothy, respectively, showed the alfalfa soil to nitrify more rapidly than the timothy soil, both in the soil on which the crops had been grown continuously and in that from which they had been removed and on which the soil had been kept bare for two seasons. The formation of nitrates was in the same order when the soils were incubated with dried blood. It seems probable that the character of the organic matter left in the soil by the plants determines to some extent the rate of nitrate formation in this soil.

Plats of land planted to certain crops in 1910 were kept bare of vegetation during the early part of the growing season of 1911. Determinations of nitrates in the soil of these plats showed a distinct and characteristic relation of the several plants to the nitrate content of the soil in the year following that in which the plants were grown.

Maize was the only crop following which the nitrates in the previously planted soil were higher than in the unplanted soil. Potato soil was the next highest in nitrates, and oat soil contained least nitrates.

Millet planted on these plats on July 1 was markedly influenced by the previous crops, but the luxuriance of growth was inversely proportional to the nitrate content of the plats.

In this case the beneficial influence of a crop on a succeeding crop was not to be attributed to the favorable influence exerted on nitrification, but this would doubtless differ with different soils; and if this effect of certain plants on nitrate formation in the following year should be of general application, the influence of a crop on nitrification may be an important factor in crop rotation.

Freezing and thawing produced a condition of soil favorable to nitrate formation. The well-known action of frost in producing a granular condition in a clay soil may have operated to some extent in accomplishing this. To what extent the greater activity of the nitrate-producing bacteria is due to aeration and how much it is due to the direct effect of cold in readjusting the equilibrium of the bacterial flora, it is impossible to say. The obvious effect is to overcome the depressing influence of the crop previously grown and to permit the resumption of nitrate formation.

Timothy maintained a lower nitrate content in the soil than did any other crop. Mixed grasses — *Phleum pratense, Agrostis alba, Poa pratensis* — gave much less nitrogen in the crop and the drainage water combined than was contained in the drainage water from unplanted soil. These facts indicate a strongly repressive influence of these plants on nitrate formation, and suggest a possible cause for the injurious effect of grass sod in orchards on soil in which the supply of available nitrogen is deficient.

Plants of two different kinds were grown, in combination and separately, in soil and in ground quartz containing nutrient solutions. In a considerable number of cases in which the plants were harvested at the blooming period or before, one or both kinds made a larger growth in the combinations than in the pure cultures, although there were at least twice as many plants growing in the mixed as in the pure cultures. This increased growth of one plant in association with another was apparently not due to any increased supply of nitrates, since the apparent stimulation of growth occurred with nutrient solution in which all the nitrogen was in the form of nitrates, as well as with the soil.

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The writers wish to express their obligation to E. W. Leland, who has had charge of the field plats during nearly the entire time that these experiments have been in progress and whose careful execution of that part of the work has contributed much toward the accuracy of the investigation.

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CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

THE ACTION OF CERTAIN NUTRIENT AND NON-NUTRIENT BASES ON PLANT GROWTH

I. The Antitoxic Action of Certain Nutrient and Non-Nutrient Bases With Respect to Plants

II. The Toxicity of Manganese and the Antidotal Relations Between This and Various Other Cations With Respect to Green Plants

III. Toxicity of Various Cations

BY M. M. McCOOL
OF THE DEPARTMENT OF PLANT PHYSIOLOGY

ITHACA, NEW YORK
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THE ACTION OF CERTAIN NUTRIENT AND NON-NUTRIENT BASES ON PLANT GROWTH

THE ACTION OF CERTAIN NUTRIENT AND NON-NUTRIENT BASES ON PLANT GROWTH — I

THE ANTITOXIC ACTION OF CERTAIN NUTRIENT AND NON-NUTRIENT BASES WITH RESPECT TO PLANTS *

M. M. McCool

(*Received for publication July 1, 1912*)

In determining whether any substance or ion may act injuriously on plant growth, it is customary to compare the rate or amount of growth in the presence of such a substance with that in its absence. The presence of any chemical agents aside from the substance tested is a complication to be avoided, or at least controlled, if possible, since there may be either a direct reaction between the different substances in the substratum or an indirect influence exerted through changes induced in the plant. In testing any injurious action — such as inhibition or retardation of growth — of the mineral nutrients used singly in solution, it is important in the first place to compare the growth-rate with that of plants in pure distilled water. The distilled-water comparison is not ideal, but it is necessary. Further comparisons may be made with plants grown in full nutrient solutions. In the hands of various investigators this type of controlled experiment has yielded results of much value, and such results may be made useful in studying the more complex conditions existing in the soil.

REVIEW OF GENERAL LITERATURE

While making a study of the proper composition of nutrient solutions, Von Raumer (1883)† reported the observation that a solution containing magnesium without calcium is highly injurious to plants. Loew (1892a) reported the first extensive study of the function of these two nutrients and of a relation between them with respect to plants. In this valuable paper, which has been overlooked by some of the later investigators, Loew emphasizes two most important points: (1) In a nutrient solution lacking calcium the organism *Spirogyra* is rapidly injured, the injury being more rapid than when any other nutrient is omitted. (2) Although magnesium is an essential mineral nutrient it is extremely injurious when there is no calcium in the culture solution, the presence of calcium in sufficient amount entirely preventing the toxic action of magnesium.

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* Laboratory of Plant Physiology, Cornell University, Contribution No. 9.

†Dates in parenthesis refer to bibliography.

Loew regarded the toxic action as due to the displacement of calcium by magnesium in certain parts of the protoplast, with a consequent loss of function. Moreover, if sufficient quantities of calcium salts are placed at the disposition of the plant thus affected, then, in accordance with the law of mass action, the magnesium is again replaced by calcium and the normal equilibrium is restored. There is here a clear enunciation of the physiological balancing of magnesium by calcium. Loew appears to have been unaware of the earlier work on antagonistic action, in which certain physiological processes of animals or their effects on animal life were under investigation. However, since Loew studied plants, and since he developed one of the most important of the antagonistic relations — that between calcium and magnesium — his work may be made the starting point in this study.

Extensive data have been presented since 1899 concerning the antagonistic action of different bases with respect to both animals and plants. The work of Loew, Kearney, Loeb, Harter, Osterhout, and others has developed many facts respecting this phenomenon in general. It has been satisfactorily demonstrated that a solution of any one nutrient mineral base at some concentration below the limits of osmotic influence is commonly poisonous. (Some plants are apparently uninjured by calcium chlorid at concentrations approaching the point of plasmolysis.) Furthermore, this poisonous action, or toxicity, can be more or less completely counteracted, sometimes by one base but especially by such a combination of salts as enters into a balanced nutrient solution.

It will be the purpose of a later paper to summarize the data on the relative toxicity of the different bases, and to present some additional facts. The present discussion is confined to the question of the extent and significance of antitoxic action between certain bases. Further studies on this subject with some of the more toxic metals will also be reported later.

The work of Loew (1892 a), above referred to, established in an incontrovertible manner the antagonistic action of calcium and magnesium, but from this there are afforded no special indications that further antidotal relations may exist between other mineral bases. Indeed, Loew subsequently declared that between other pairs of mineral nutrients no relations of the same class occur.

In studying the effects on plants of certain salts of alkalin soils, Kearney and Cameron (1902) greatly extended the knowledge of toxic and antagonistic effects. Some of their conclusions are the following:

" Calcium chloride in pure solution is ten times less injurious than sodium chloride, and two hundred times less injurious than magnesium sulphate, if chemically equivalent solutions are considered.

"Addition of sodium ions to a solution containing magnesium ions in most instances markedly weakens the toxic action of the latter.

"Addition of calcium ions to solutions containing either sodium or magnesium ions nearly always counteracts to an extraordinary degree the injurious effect of the sodium or magnesium ions, this beneficial influence being usually much more marked when calcium is furnished as the sulphate than when the chloride is added.

" The effect of one kind of ion in counteracting the physiological action of another kind can not be entirely explained by a study of the chemistry of the solution itself, but must in part be referred to complicated changes in the protoplasm of the organisms. The theory that ions furnished by the dissociation of electrolytes form intimate combinations with the proteids of protoplasm, and that their mutually antagonistic effect expresses itself in a replacement of one kind of ion by another as a result of change in the composition of the surrounding solution, would appear to afford the key to this problem."

The results from one of the more general studies undertaken by Osterhout (1906) may be noted in this connection. These conclusions are important, although at the time Osterhout was apparently unaware of the earlier work on plants. He placed several forms of marine algæ in pure and mixed salt solutions and determined the duration of life in days. The following conclusions are drawn:

" 1. Each of the salts of the sea water is poisonous where it alone is present in solution.

" 2. In a mixture of these salts (in the proper proportions) the toxic effects are mutually counteracted. The mixture so formed is a physiologically balanced solution.

" 3. Such physiologically balanced solutions have the same fundamental importance for plants as for animals."

In studying the toxicity of sodium and magnesium salts, Kearney and Harter (1907) placed seedlings in salt solutions for twenty-four hours and then transferred them to pure water. The inability of the roots to elongate further was taken as an indication that the concentration used was fatal to the root tips. Kearney and Harter made studies of the lethal concentrations of pure solutions and of solutions with different amounts of calcium sulfate added, employing as indicators a number of species of plants. They found that the addition of calcium sulfate greatly diminishes the toxicity of the sodium and magnesium salts to all the plants tested.

Among the many experiments bearing on this problem reported by Loeb (1905 a) the following may be mentioned: The marine fish *Fundulus* soon dies when placed in a pure solution of sodium chlorid. This is true even though the solution may be no stronger than that which exists in sea water. The toxicity of the sodium chlorid is counteracted by the addition of chlorids of calcium or potassium. The calcium and potassium serve primarily to counteract toxicity, and in this instance are unimportant as nutrient elements; for when the fish is placed in distilled water it lives for a considerable period of time.

The brief indications given above may serve to show the direction and importance of the work thus far undertaken. On the whole, many facts have been presented concerning the antagonistic action of different bases with respect to both animals and plants. Nevertheless, the data for the seed plants are still insufficient, the evidences of slightly unbalanced conditions on the development of the plants as a whole having received much less attention. With respect to unbalanced conditions in the soil the problems become most complex, and solution studies should make possible a more nearly accurate approach to the agricultural application.

The more recent work of Osterhout (1908, 1909) with wheat seedlings placed in pure and mixed salt solutions is important, but much more data are required. His conclusions also are based on a comparison of root growth alone. Furthermore, it cannot be seen from his tables, in many cases, to what extent one base antidotes another base. For example, the root growth of wheat seedlings placed in solutions containing 100 cubic centimeters of .12 mol.* NaCl + 100 cubic centimeters of .12 mol. NH_4Cl (the resulting concentrations of each salt being .06) is compared with the root growth of those placed in pure solutions of .12 mol. NaCl and .12 mol. NH_4Cl, respectively. The increase in growth of the roots in the mixed solutions over that in the pure cultures is attributed wholly to the antagonistic action of the bases sodium and ammonium, whereas it is certainly due in part merely to the reduction of the concentration of each salt from .12 to .06 mol.; yet antagonism is obvious.

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* As here employed, " mol. " denotes a gram-molecular solution, that is, one containing the number of grams corresponding to the molecular weight of the substance dissolved to make 1 liter of solution.

In view of all the considerations stated, a reinvestigation of antitoxic action with reference to certain seed plants has seemed especially necessary; likewise, it is important to extend the work so as to include certain bases that have been less studied.

SCOPE OF THE INVESTIGATION

The writer has concluded experiments with various nutrient and non-nutrient mineral bases, using Canada field peas and wheat as indicators. He has made a comparative study of the growth of tops and roots in solutions of the different salts containing single bases, in concentrations varying from those that are non-toxic to those that practically prohibit growth, and in solutions containing two bases at various concentrations including those strengths that are toxic when employed alone. An effort was also made to determine the minimum of one base that would antidote a given toxic amount of another base, but much remains to be done in this direction.

The experiments reported in the present bulletin include studies of the bases Ca, K, Na, NH_3, Mg, Sr, and Ba. In studying the relations of calcium and strontium ions they were combined as $Ca(NO_3)_2 + Sr(NO_3)_2$ and $CaCl_2 + SrCl_2$. The experiments with calcium and magnesium consisted of $CaCl_2 + MgCl_2$ and $CaCl_2 + MgSO_4$. In all other cases the chlorids only were used. Data which are to be presented show that calcium antidotes each of the bases mentioned above, and that definite mutual antagonism exists in the following combinations:

Mg and Sr
Mg and Ba
Na and K
Na and NH_3
Na and Sr
K and Sr
K and Ba

Other cases in which mutual antagonism may possibly occur are discussed later.

METHOD

Analyzed salts of high quality were used in all experiments. The normal (equivalent) solution was made the basis of comparison. A normal solution of a monovalent salt contains one gram molecule in a liter of solution; while a normal solution of a bivalent salt contains one half gram molecule in a liter. In general, stock solutions of N/1 were made and the desired concentrations were subsequently prepared by dilution. In some of the experiments wheat seedlings were used, in others Canada field peas. The seeds were treated for fifteen minutes with a solution of formaldehyde containing one part of the toxic agent in six hundred parts of water. Following this treatment the seeds were left in running water for twenty-four hours and were then allowed to germinate between filter papers over sphagnum or peat moss saturated with water. In this way entirely satisfactory seedlings were obtained.

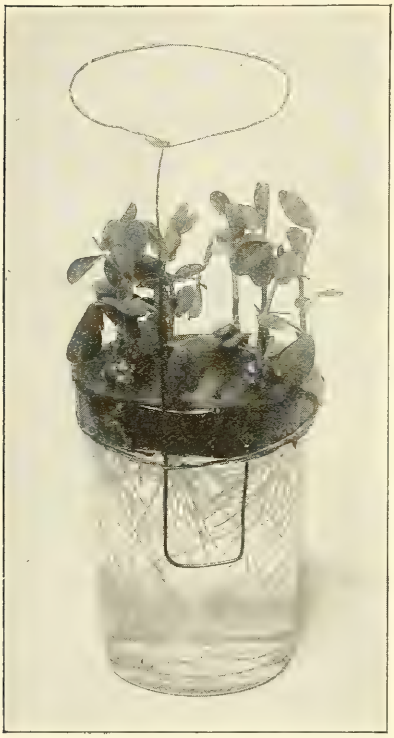

Fig. 1.— *Showing method of making cultures. The black paper shell has been removed in order to expose the roots*

The seedlings, after having been washed with distilled water, were transferred to the culture solutions when the radicles were about two inches in length. At this stage of development there was no lateral branching of the roots. Seedlings of as nearly uniform size as possible were chosen for the cultures.

All glassware used in the experiments was carefully washed with the chromic-acid cleaning mixture and then rinsed with tap water and distilled water. Ordinary glass tumblers were used as culture vessels. Preliminary experiments were made in order to determine whether or not there is any appreciable difference in the growth of plants in distilled water and in various solutions when resistant glass beakers are substituted for ordinary glass tumblers as containers of the culture solutions. The results showed conclusively that the differences are not recognizable. The use of the tumblers as receptacles for the solutions is thus justified. In order to support the seedlings and to prevent evaporation the tumblers were covered with paraffined wrapping paper, made secure by means of rubber bands. Holes were made in the paper and the radicles were inserted through these into the solutions below. The tumblers were also slipped into black paper shells.

If the tops of the seedlings grew to a height of three inches they were supported by means of wires carrying rings which projected directly above the tumblers. These wires held the tops of the plants upright and thus afforded uniform conditions of light.

The duration of the experiments was twenty-one to thirty days. Frequent observations were made until the end of the period, when generally the final notes were taken. The original amount of the solution was maintained by the addition of distilled water. The writer has found this method satisfactory. In all cases the essential data are given in the tables or in the text in connection therewith.

RELATION BETWEEN CALCIUM AND MAGNESIUM

Review of important field work

The calcium-magnesium relation of soils has been shown to be a most important one in crop production. Previous to the formulation of the view that there is a definite lime-magnesia ratio for maximum plant growth, many contradictory reports existed respecting the benefits or injuries from the application of lime and magnesia to soils.

As early as 1814, Davy (1814) discussed the injury that magnesia sometimes produces on crops. He wrote, " On mixing some calcined magnesia with soil in which different seeds are sown, it is found that they either die or vegetate in a very imperfect manner." He also states " that lime from magnesia limestone may be applied in large quantities to plots, and where lands have been injured by the application of too large quantities of magnesia lime, peat will be a proper and efficient remedy."

The deleterious influence of a high magnesium lime was reported by the United States Commissioner of Agriculture in 1876. Parts of a field fertilized in one case with material obtained from a place near Belvidere, New Jersey, and in another case with material obtained from Oxford, New Jersey, showed injurious effects in the former, and beneficial action in the latter, on the crop growth. The difference between the two reactions was so striking that it was considered of importance to investigate the cause. Samples of the two limestones were sent to the United States Department of Agriculture, where they were analyzed. The injurious limestones were found to contain thirty-eight to forty-two per cent of magnesium carbonate, while the beneficial limestones contained not quite one per cent of this substance.

Adolf Mayer (1886) mentions unproductiveness as characteristic of soils rich in magnesia. On the other hand, Heiden (1869) cites some instances in which magnesia, and even magnesium sulfate, were beneficial when used as fertilizers on soil.

There are conflicting reports regarding the value of kainit and carnallite as fertilizers under different conditions. Kainit is composed of K_2SO_4, $MgSO_4$, $MgCl_2$, and water of crystallization. Carnallite is a double chlorid of potassium and magnesium. The effects were frequently found to be injurious when the salts were applied in spring, while the application in autumn proved beneficial. Thus Fleischer (1886) observed that when kainit was applied in autumn the yield of a crop of potatoes was five per cent greater than when kainit was applied in spring. Liebenberg (1896) reported a decrease in the yield of meadows when kainit was applied in autumn. In the latter case the perennial roots of grasses came in direct contact with the fresh fertilizer; while in the previous cases winter rains washed out or modified the injurious magnesium salt before seeds were sown.

The detrimental action of these salts is due mainly to their high magnesia content — which will do little harm on soils rich in lime and poor in magnesia. The more magnesia there is present in a soil, in relation to lime — other conditions being constant — the more injurious a certain additional quantity of magnesium compounds will prove.

It is thus evident, from these general experiments, that there are contradictory reports on the results from the application of various amounts of lime and magnesia to the soil. If it be assumed, however, as is permissible from data subsequently mentioned, that there is a certain favorable ratio between lime and magnesia, and particularly an injurious action from an excess of magnesium, then these reports are not often inconsistent, especially when cognizance is taken of the widely different ratios of lime and magnesia in different soils and of other complex relations.

The composition of soils can be determined by means of chemical analyses, but such analyses do not reveal the ratio in which the various mineral nutrients are available to the plant. The elucidation of fundamental principles from soil studies is very difficult because of the many complex and uncontrollable factors existing therein.

Loew (1901), in a review of the ratio between lime and magnesia in soils of different countries, states:

" It will be seen from this review,

" 1. That the ratio of lime to magnesia ranges between wide limits.

" 2. That in the majority of cases lime predominates over magnesia.

" 3. That in all the instances of great fertility the soil never shows any marked excess of magnesia over lime, but, on the contrary, generally more lime than magnesia."

He writes also: " Lime and magnesia can exert their indispensable nutritive functions only in a certain dependence upon each other. Hence a certain ratio between these two nutrients will produce the most favorable results, while a great excess of the one in the finest portion of the soil will lead to starvation and of the other to poisonous phenomena." It is therefore clear that the determination and balancing of the available amounts of magnesia and lime in the soil is necessary for successful farming on apparently infertile soils.

It was in attempting to explain the relation of calcium to magnesium that Loew (1892a) postulated the existence, in plants requiring calcium, of a calcium-protein compound in which the base is not replaceable by magnesium without serious injury to the protoplasm.

According to Sirker (1908), " a topdressing with a small quantity of magnesium sulphate at the rate of 10 kilo per hectar had the favourable effect of increasing the harvest by 31 per cent on a plot too rich in lime relatively to magnesia."

Review of some culture experiments

Investigations with oats, wheat, cowpeas, and tobacco, in water, sand, and soil cultures, were made by May (1901). The following general conclusions were reached:

"Magnesia in a soil in great excess over lime in a finely divided or soluble condition is noxious to the growth of plants. With a great excess of lime over magnesia the physiological action of the plant is hindered and it exhibits phenomena of starvation. An excess of lime counteracts the poisonous effects of magnesia, while the more favorable proportion of the two bases obviates the poor nutrition of the plant.

"The best proportion of soluble lime to soluble magnesia for the germination and growth of plants is about molecular weight 5 to 4, or actual weight 7 to 4.

"In liming soils the amount of lime and magnesia should be first determined in both the soil and the material applied. In this way only can the process be intelligently carried out and the best ratio between the two bases for the promotion of the growth of crops be maintained."

Meyer (1901) grew oats, potatoes, horse beans, vetch, and a mixture of equal amounts of rye grasses and alfalfa in pots of a capacity of six kilos, which contained, on the one hand, sand and peat ($2\frac{1}{2}$ per cent) and, on the other hand, sand and loess loam (10 per cent).

In the case of the oats-and-grass mixture, an application of gypsum at the equivalent rate of more than one gram CaO per pot caused a decided reduction of yield. The injurious effect of the gypsum was overcome by applications of calcium carbonate or magnesium carbonate.

Large applications of magnesium carbonate depressed the yield of the grass mixture. It was observed also that even when an excess of lime had been applied magnesia caused an increased yield.

With pot experiments Aso (1903) found that the ratio of lime to magnesia for the mulberry tree is about two or three parts of lime to one part of magnesia. An excess of magnesia over lime retards growth. In some experiments with rice Aso (1904) used in pot cultures soil that was taken directly from a paddy field. To these cultures were added varying amounts of calcium carbonate and magnesite. From the results obtained with this crop Aso concludes that the ratio of lime to magnesia for maximum growth is about the same as that most favorable for other *Gramineæ,* which is between 1:1 and 2:1.

Kanomata (1908) found that "when the amount of lime is increased in undue proportion to the amount of magnesia present, the yield of oats is considerably depressed. In sand culture, there was a decrease by 39 per cent of the weight of shoots before flowering time, when the amount of limestone and magnesite differed so much that the ratio $\frac{CaO}{MgO}$ was changed from 1/1 to 100/1." This last point, however, does not appear to the writer to be established through sufficient experiments.

Daikuhara (1905 a) observed that "the yield of naked barley was not essentially altered when the amounts of these bases [calcium and magnesium] were rendered equal by adding $CaCO_3$ or $MgCO_3$," when the difference did not exceed .5 per cent. On well-manured soils in which the ratio was .34:1 the harvest was doubled by producing a ratio of 1:1.

The data obtained by the same author (1905 b) from sand cultures show that "in the presence of lime as carbonate the necessary amount of magnesia when applied as crystallized sulphate for paddy rice in sand culture is so small that the best ratio CaO:MgO becomes 30:1, while in the form of natural carbonates the best ratio would be 1:1."

The results given by Nakamura (1905) with barley grown in pots of soil "show decidedly that the addition of a certain quantity of magnesia acts very beneficially upon the growth of the plant when the soil contains a large excess of lime over magnesia and furnish at the same time a further proof of the inference that 'a maximum yield depends — other things being equal — also upon a certain ratio of lime to magnesia which enters into the plant.'"

Wheeler and Hartwell (1903) found that in pot cultures $CaCl_2$ and NH_4Cl exert "a marked poisonous action upon certain plants, when applied to a soil which was already somewhat acid. Magnesium chlorid was not found to be poisonous under conditions where great injury from calcium chlorid and ammonium chlorid resulted.

"Calcium carbonate and caustic magnesia used singly, also a mixture of basic slag meal with the carbonates of potash and magnesia, were found to prevent or overcome the ill effect produced by applications of either calcium chlorid or ammonium chlorid."

Bernardini and Siniscalchi (1908) grew lupine in pot cultures and concluded that "the injurious action of an excess of lime and the poisonous action of an excess of magnesia in the soil is not due to the absolute quantity of calcium and magnesium ions absorbed by the plant, but to the ratio in which they are absorbed."

Konovalov's results (1907) with wheat, lupine, and oats grown in water and sand cultures do not confirm Loew's view that "there is a definite lime-magnesia ratio for each plant." The growth of the plants is injuriously affected when magnesia is present and lime is absent in nutrient solution. According to Konovalov the yield is increased with the increase of the proportion of lime to magnesia.

Culture experiments with rye, corn, and kidney beans are reported by Bernardini and Corso (1908). The nutrient solution contained the ratios of lime to magnesia (magnesia as 1) 3, 2, 1, $\frac{1}{2}$, and $\frac{1}{3}$. Rye gave the best result in the solution in which the relation of lime to magnesia equalled 1, and better results with the higher proportions than with the lower; corn gave the best result with the proportion of lime to magnesia equal to 2, and better with the higher than the lower, showing that an excess of lime does less harm than an excess of magnesia. In the pot experiments a soil of known composition was used and the proportions between lime and magnesia were made the same as in the nutritive solution experiments, and the same results were obtained with rye and corn; with kidney beans the proportion of lime to magnesia equal to 3 gave the best result.

Konovalov (1909) studied the ratio of $\frac{CaO}{MgO}$ for barley, millet, oats, and maize. He states:

"In sand cultures with oats and millet the ratios of lime and magnesia varied as follows: $\frac{13.4}{1}, \frac{6.7}{1}, \frac{3.3}{1}, \frac{1.6}{1}, \frac{0.8}{1}, \frac{0.4}{1}, \frac{0}{1}$.

"The data clearly show that the more lime there is in the nutritive solution the quantity of magnesia remaining the same, until the ratio of lime to magnesia becomes 6.7:1 the greater the yield of both millet and oats. When this ratio is exceeded the yield of millet decreases somewhat and that of oats very considerably, there being a falling off in yield of both grain and straw and the mean weight of the grain."

In water cultures with maize and barley, "the maximum yield of organic matter was obtained in case of barley when the ratio of lime to magnesia was 3.3:1 and in case of maize when the ratio was .8:1. The same ratio gave the maximum yield of above ground material."

Novel experiments with wheat and oats were conducted by Hansteen (1909). He inserted one of the primary roots of the same seedling into each of two glass tubes placed side by side. The tubes contained separate solutions; for example, in tube 1 was placed N/50 $Ca(NO_3)_2$, while tube 2 contained N/50 $Ca(NO_3)_2$ + N/50 $MgSO_4$. In this manner Hansteen was able to determine the influence of various solutions on different roots of the same plant. The results substantiate the view that $Ca(NO_3)_2$ mollifies the deleterious properties of $Mg(NO_3)_2$ and $MgSO_4$. Furthermore, in solutions that contain no calcium, only slight elongation of the roots of wheat and oats takes place.

From experiments with flax grown in pots of soil in which the ratios $\frac{CaO}{MgO}$ were 1:1 and 2:1, respectively, Namikawa (1906) concluded that the most advantageous ratio of $\frac{CaO}{MgO}$ for flax is 1:1. The yield is 21 per cent lower in soil cultures that have the proportions of lime to magnesia 2:1 and 3:1. Similar results were obtained with spinach grown in sand cultures.

Maki and Tanaka (1906) grew barley to maturity in loamy and sandy soil cultures. Sufficient slaked lime was added to make the ratio $\frac{CaO}{MgO}$ about 3:1. This relation caused a decrease in the yield, or the soil was overlimed. When a sufficient quantity of magnesium sulfate was applied to make the ratio 1:1 in the overlimed soil, the harvest was almost as great as in the untreated soil. In case of the sandy soil the crop was greater than the control cultures.

Yokoyama (1908) observed that when oats are grown in pots containing sandy soil poor in lime and magnesia the addition of lime is injurious, due to the unbalanced ratio of lime to magnesia. Furthermore, such soils should be treated with dolomitic limestone in order to correct such unbalanced conditions.

In sand cultures Aso (1909) ascertained that the lime factor for oats is 1, for adzuki beans (*Phaseolus mungo*) about 2, for rice 1, and for Italian millet between .5 and 1. His conclusions are:

" 1. Certain favorable ratio of lime to magnesia for plant-growth exists even in sandculture.

" 2. Absolute excess of lime or magnesia provided it be kept within certain limits, has no retarding effect on the development of the plants, the ratio between these bases being the chief factor for plant growth."

Gile (1913), as a result of his work, states: " The toxicity of an excess of lime or magnesia is not due simply to an unfavorable ratio between these two salts alone, but to an unfavorable proportion between the salt which is in excess and all the other salts present."

Experimental work

In the experiments from which the data presented in this section were taken, the sulfates and chlorid of magnesium were used but only the chlorid of calcium; that is, in one series $CaCl_2$ and $MgCl_2$ were employed, in another $CaCl_2$ and $MgSO_4$. The different salts of magnesium were used in order to determine whether or not the acid radical is of importance in these studies.

A study of Table 1 shows that the chlorids and sulfates of magnesium are extremely toxic to pea seedlings. On the other hand, calcium chlorid, even at relatively high concentrations, is not poisonous. The development of the plants in the combined solutions is much greater than in pure solutions of magnesium chlorid or sulfate. The growth of the plants in the combined cultures does not, however, differ greatly from that of the plants in the solution of the calcium salt alone; for example, in N/20 $CaCl_2$ the total weight of the plants is 7.40 grams, in N/500 $MgCl_2$ the weight is practically nil, and in N/20 $CaCl_2$+N/500 $MgCl_2$ the total weight is 9.95 grams.

TABLE 1. EXPERIMENT WITH CANADA FIELD PEA. DURATION, TWENTY-FOUR DAYS. DATA FOR TEN PLANTS

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots[*] (centimeters)
N/20 $CaCl_2$+N/20 $MgCl_2$	5.70	4.00	9.70	12.0	12.5
N/20 $CaCl_2$+N/100 $MgCl_2$	6.65	4.25	10.90	14.5	15.0
N/20 $CaCl_2$+N/500 $MgCl_2$	6.10	3.85	9.95	13.0	14.0
N/100 $CaCl_2$+N/20 $MgCl_2$	5.85	3.00	8.85	12.0	11.0
N/1,000 $CaCl_2$+N/20 $MgCl_2$	1.70	1.40	3.10	4.5	5.0
N/1,000 $CaCl_2$+N/100 $MgCl_2$	8.60	4.20	12.80	17.0	10.0
N/1,000 $CaCl_2$+N/200 $MgCl_2$	6.60	3.00	9.60	14.0	10.0
N/20 $CaCl_2$	5.00	2.40	7.40	8.5	12.0
N/100 $CaCl_2$	5.70	2.75	8.45	11.5	8.5
N/300 $MgCl_2$	—[†]	—[†]	—[†]	—	—
N/500 $MgCl_2$	—[†]	—[†]	—[†]	2.0	—
Distilled water	1.85	1.75	3.60	6.0	6.5
N/20 $CaCl_2$+N/20 $MgSO_4$	6.24	4.30	10.54	11.5	12.0
N/20 $CaCl_2$+N/100 $MgSO_4$	7.14	5.12	12.26	15.0	14.0
N/20 $CaCl_2$+N/500 $MgSO_4$	7.00	4.12	11.12	13.0	13.5
N/20 $CaCl_2$	5.85	2.60	8.45	9.0	12.5
N/500 $MgSO_4$	—[†]	—[†]	—[†]	3.0	—
Distilled water	1.72	1.84	3.56	6.5	7.0

[*] The average length of roots was determined by measuring the main roots. The lateral roots were not measured, as the weight is indicative of their development. These statements apply to all subsequent tables.

[†] Weak growth, plants almost dead, not weighed.

The action of the calcium is a very clear case of antagonism. It entirely overcomes the toxicity of both chlorid and sulfate of magnesium. It appears that the magnesium antidotes the calcium to a slight extent. This apparent antagonism, however, may be merely a benefit arising from the addition of another essential element, or at least it may not be due wholly to the antitoxic action of the magnesium. It is difficult to interpret mutual antagonism from data that show great decrease of toxicity in one direction and only slight decrease in the other.

Five days after the seedlings had been placed in the solutions the toxicity of magnesium was apparent. The roots in the culture containing N/500 $MgCl_2$ were dead, no growth having taken place. Ten days later the antagonism of calcium was clearly shown by the increased growth of the seedlings in the combined solutions over those in the solutions of magnesium alone.

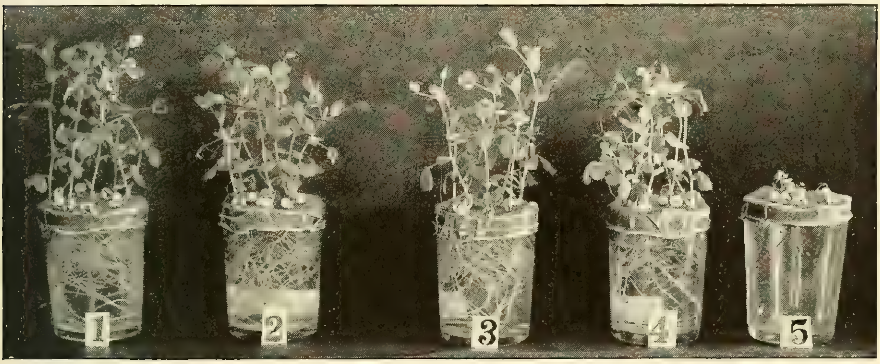

FIG. 2.— *Antidotal relations between calcium and magnesium; distilled water as the solvent*

1. N/20 $CaCl_2$
2. N/20 $CaCl_2$ + N/100 $MgCl_2$
3. N/100 $CaCl_2$ + N/100 $MgCl_2$
4. N/1000 $CaCl_2$ + N/100 $MgCl_2$
5. N/100 $MgCl_2$

Loew (1899) has drawn attention to the relative abundance, usually, of magnesium in the seeds of plants as contrasted with the magnesium content in other plant structures. This excess in the seed may enable the seedling, for a time at least, to exhibit a maximum growth in a solution containing no magnesium. As a result of numerous nutrition experiments in this laboratory with the pea, it has been clearly demonstrated that the demand of this plant for magnesium, or the necessity of maintaining a certain proportion of magnesium in cultures with calcium salts or with full nutrient solutions (lacking magnesium), is unimportant during the first two weeks of growth. These results are in agreement with those obtained by König and Haselhoff (1894). These investigators found that *Leguminosæ* endure the lack of potassium better than the lack of calcium.

Numerous data have been brought forward which seem to show that a more or less definite ratio of calcium to magnesium is beneficial for plants. Many of the reports in which these data are embodied have been summarized. A further study of the antidotal relations that exist between these bases in full nutrient solutions is interesting and important. Canada field pea seedlings were grown in nutrient solution cultures containing calcium and magnesium in various proportions. The nutrient solution that was used as the solvent for these bases in series 1 was made up as follows:

$Ca(NO_3)_2$	4.00 grams
KH_2PO_4	1.00 gram
KNO_3	1.00 gram
$MgSO_4$	1.00 gram
KCl	0.50 gram
$FeCl_3$	0.01 gram
Distilled water	3.00 liters

No considerable antagonistic action is evident from cultures made up as were those in this series, inasmuch as the quantities of calcium and magnesium employed are not to be considered toxic under the conditions. However, a slight increase of growth ensues when either calcium or magnesium is added to the nutrient solution, but in general this is not so extensive as in the cultures to which both are added. The data obtained from this set of experiments appear in Table 2.

In this table toxicity is not evident, although the addition of calcium chlorid alone or calcium and magnesium together causes increased growth over the control (full nutrient alone). It may be that the full nutrient solution (Pfeffer's) is not a properly balanced solution for Canada field pea.

The nutrient solution employed in the second series of experiments (Table 3) was one tenth as strong as that in the preceding cultures.

TABLE 2. SERIES 1. EXPERIMENT WITH CANADA FIELD PEA. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution (nutrient solution as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)	Color of tops	Remarks
N/25 $CaCl_2$ + N/50 $MgCl_2$....	12.85	9.00	21.85	20	14	Light at tips	Lateral roots good length
N/25 $CaCl_2$ + N/100 $MgCl_2$...	14.15	8.25	22.40	20	12	Light at tips	Lateral roots good length
N/25 $CaCl_2$ + N/200 $MgCl_2$...	14.30	8.85	23.15	19	12	Good	Lateral roots good length
N/100 $CaCl_2$ + N/100 $MgCl_2$...	10.25	6.50	16.75	14	12	Good	Lateral roots medium length
N/500 $CaCl_2$ + N/100 $MgCl_2$...	8.45	4.60	13.05	12	10	Good	Lateral roots fair
N/1,000 $CaCl_2$ + N/100 $MgCl_2$...	10.70	5.60	16.30	15	14	Good	Lateral roots short
N/50 $MgCl_2$.....	10.55	5.90	16.45	15	12	Good	Lateral roots short
N/100 $MgCl_2$....	9.65	5.50	15.15	14	11	Good	Lateral roots medium
N/200 $MgCl_2$....	10.40	5.70	16.10	11	9	Good	Lateral roots medium
N/25 $CaCl_2$......	11.85	6.65	18.50	20	14	Pale green	Lateral roots medium
N/100 $CaCl_2$.....	13.50	7.90	21.40	17	9	Light green	Lateral roots good
Nutrient solution.	10.40	5.20	15.60	13	10	Good	Lateral roots fair

Toxic strengths of magnesium were used in these experiments. It is to be noted that in this nutrient solution N/10 $MgCl_2$ prevents growth of roots, and solutions of N/50 are injurious. The leaves of the plants grown in solutions of N/10 $CaCl_2$ are pale green in color, and those in N/100 $CaCl_2$ are somewhat lighter than in the control cultures.

The toxicity of N/10 $MgCl_2$ (Table 3) is greatly reduced, although not entirely prevented, by the same concentration of calcium chlorid. It is also to be noted that the harmful action of either N/20 or N/50 $MgCl_2$ is completely counteracted by solutions of N/10 $CaCl_2$. No injury results from the magnesium in cultures composed of this nutrient solution + N/100 $CaCl_2$ + N/50 $MgCl_2$.

In this series of experiments the addition of magnesium to the solutions of calcium has not resulted in an appreciable increase in the growth of the plants. It is to be observed, however, that the chlorotic condition induced by the calcium is prevented by the magnesium. A summary of the data obtained appears in Table 3.

TABLE 3. SERIES 2. EXPERIMENT WITH CANADA FIELD PEA. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution (1/10 nutrient solution as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Color of tops	Remarks
N/10 $CaCl_2$+N/10 $MgCl_2$	5.12	2.65	7.77	Dark green	Lateral roots very short
N/10 $CaCl_2$+N/20 $MgCl_2$	6.15	3.75	9.90	Dark green	Lateral roots very short
N/10 $CaCl_2$+N/50 $MgCl_2$	5.77	4.37	10.14	Light green	Lateral roots very short
N/10 $CaCl_2$+ N/100 $MgCl_2$	4.55	3.47	8.02	Light green	Lateral roots good development
N/100 $CaCl_2$+ N/50 $MgCl_2$	6.57	4.27	10.84	Green	Lateral roots good development
N/10 $CaCl_2$	5.00	4.10	9.10	Pale green	Lateral roots good development
N/100 $CaCl_2$	6.80	4.25	11.05	Light green	Lateral roots good development
N/10 $MgCl_2$	Weight not taken	No growth	———	———	Practically dead
N/50 $MgCl_2$	4.86	1.75	6.61	Dark green	Lateral roots short, dead
N/100 $MgCl_2$	6.00	3.75	9.75	Dark green	Lateral roots poor growth
1/10 nutrient solution	5.85	3.55	9.40	Green	Lateral roots good

RELATIONS BETWEEN OTHER BASES

Potassium and magnesium, sodium and magnesium

When wheat seedlings are placed in solutions of potassium and magnesium or sodium and magnesium chlorids (distilled water being the solvent), mutual antagonism is evident. In Table 4 it may be noted that N/1000 KCl can antidote the toxicity of N/500 $MgCl_2$ as completely as can N/50 KCl; while N/500 $MgCl_2$ seems to be the best concentration for antidoting N/50 KCl. The toxic action is manifest, particularly in the development of roots.

TABLE 4. Experiment with Wheat. Duration, Thirty Days. Data for Eight Plants

Composition of solution	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 KCl	0.65	5.5	1.0
N/50 KCl+N/100 $MgCl_2$	0.52	4.0	5.0
N/50 KCl+N/500 $MgCl_2$	1.00	7.0	6.0
N/50 KCl+N/1,000 $MgCl_2$	0.70	6.0	4.0
N/1,000 KCl+N/500 $MgCl_2$	1.00	7.5	5.0
N/500 $MgCl_2$	Dead	4.0	Slight
Distilled water	0.56	4.0	4.0

In Table 5 a striking case of mutual antagonism is shown between NaCl and $MgCl_2$. It is remarkable that two salts when combined will permit considerable growth, when if either one is present alone it will cause death of the plant.

TABLE 5. Experiments with Wheat. Duration, Thirty Days. Data for Eight Plants

Composition of solution	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 NaCl	———*	2.0	0
N/50 NaCl+N/100 $MgCl_2$	0.400	4.0	1.0
N/50 NaCl+N/500 $MgCl_2$	0.800	5.5	4.0
N/50 NaCl+N/1,000 $MgCl_2$	0.650	5.5	2.0
N/500 NaCl+N/500 $MgCl_2$	———*	1.0	1.0
N/500 $MgCl_2$	———*	4.0	Very slight
N/50 NaCl+N/100 $MgCl_2$	0.900	8.0	8.0
N/50 NaCl+N/500 $MgCl_2$	0.975	9.0	13.0
N/50 NaCl+N/1,000 $MgCl_2$	1.125	9.0	12.0
N/100 NaCl+N/500 $MgCl_2$	1.150	11.0	10.0
N/500 NaCl+N/500 $MgCl_2$	0.840	9.0	6.0
N/1,000 NaCl+N/500 $MgCl_2$	0.810	8.0	5.0
N/500 $MgCl_2$	———*	6.0	0
N/1,000 $MgCl_2$	———*	8.0	Slight
N/50 NaCl	———*	1.0	0
N/100 NaCl	0.900	8.0	5.0
Distilled water	0.570	8.0	7.0

*Slight. Determination not made.

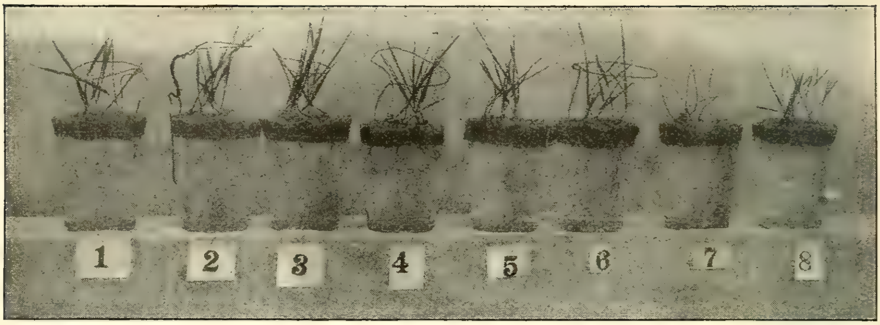

Fig. 3.— *Antidotal relations between sodium and magnesium; distilled water as the solvent*

1. N/50 NaCl+N/100 $MgCl_2$
2. N/50 NaCl+N/500 $MgCl_2$
3. N/50 NaCl+N/1000 $MgCl_2$
4. N/1000 NaCl+N/500 $MgCl_2$
5. N/50 NaCl
6. N/1000 NaCl
7. N/500 $MgCl_2$
8. Distilled water

Calcium and potassium

It has been shown by various investigators that salts of potassium are commonly much less toxic than those of magnesium. Nevertheless, a relatively weak concentration of KCl, N/50, will completely inhibit the growth of roots of the field pea; and it is only when the concentration is reduced to about N/500 that root growth is approximately equal to that when the plant is in distilled water. It has already been shown that calcium salts, at concentrations approaching the threshold of plasmolysis, may show little or no inhibition as compared with distilled water. When the salts of the two bases calcium and potassium are combined in certain proportions the toxicity of the potassium is entirely overcome, and the growth of the Canada field pea seedlings is considerably greater than in the corresponding concentrations of the calcium salt employed alone. This increase is of course partly due to the presence of another essential element. In general, the writer's results confirm and extend those of Kearney, Osterhout, Hansteen, and others, indicating that there is sufficient evidence to interpret the relation between these bases as one of mutual antagonism. Osterhout (1909), experimenting with wheat, has shown this relation as affecting root growth, and the following table includes some of the more significant of his results:

TABLE 6. WHEAT (GROWTH DURING THIRTY DAYS). QUANTITIES REPRESENT CUBIC CENTIMETERS OF .12 M SOLUTIONS

Culture solution	Aggregate length of roots per plant (millimeters)
$CaCl_2$	85
100 $CaCl_2$ 10 KCl	105
10 $CaCl_2$ 100 KCl	492
KCl	66

In the writer's experiments Canada field peas and wheat have both been employed, and in each case the presence of calcium protects the plant from the injurious action of potassium. As measured by the effect on the total green weight, the writer's results show a protective action which is not so marked as that which appears from the data of Osterhout, in which root length alone has been considered.

TABLE 7. EXPERIMENT WITH CANADA FIELD PEA. DURATION, TWENTY-FOUR DAYS

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)	Remarks
N/20 $CaCl_2$ + N/20 KCl	6.46	3.60	10.06	14.0	14.5	Tops dark green
N/20 $CaCl_2$ + N/50 KCl	6.60	3.85	10.45	15.0	15.0	Tops dark green
N/20 $CaCl_2$+N/500 KCl	6.35	3.95	10.30	14.0	15.0	Tops dark green
N/500 $CaCl_2$+N/20 KCl	5.25	3.20	8.45	12.0	8.0	Tops light green
N/20 $CaCl_2$	5.00	2.90	7.90	8.8	12.0	Tops light green
N/20 KCl	0	0	0	0	0	No growth, dead
N/50 KCl	Inap.*	0	Inap.*	Slight	0	Slight growth, dead
N/500 KCl	1.90	2.00	3.90	6.0	6.0	Terminal leaves dead
Distilled water	1.70	1.70	3.40	6.0	6.0	Color poor

* Inappreciable.

From an examination of the preceding table it is clear that a toxic solution of KCl strong enough to kill, N/20, may be rendered harmless by an equal quantity of calcium; while if the solution contains N/500 $CaCl_2$ to N/20 KCl, the maximum effect of calcium addition is not felt. On the other hand, with the maximum strength of the calcium salt employed, dilution of the KCl to N/500 does not materially affect growth as compared with that in the strongest combined concentrations employed. Inasmuch as the solutions containing calcium alone yield more growth than does the distilled water culture, the increase in growth of the plants in the combined solution over that in the solution of calcium alone should not be attributed wholly to the protective action of potassium with respect to calcium.

TABLE 8. Experiment with Wheat. Duration, Thirty Days. Data for Ten Plants

Composition of solution	Total green weight of plants (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/100 $CaCl_2$+N/100 KCl	2.65	12.5	12.5
N/100 $CaCl_2$+N/200 KCl	2.49	12.5	15.2
N/100 $CaCl_2$+N/400 KCl	2.45	12.3	15.0
N/100 $CaCl_2$	2.80	27.5	17.5
N/100 KCl	1.65	7.0	9.5
N/200 KCl	2.20	7.5	11.5
Distilled water	1.71	17.5	10.0

From a consideration of Table 8 it is seen that a solution of N/100 KCl is toxic for wheat seedlings (and it should be noted that a concentration of N/50 KCl practically prohibits growth); and this effect is to a considerable extent prevented by the addition of an equal quantity of calcium. Nevertheless, in the combined solution there is not so great a growth of tops as in the calcium alone, nor is the growth of tops in the combined solution quite so great as that in distilled water. With respect to wheat the experiments are not sufficient to determine whether or not there is a protective action of potassium toward calcium, since a toxic solution of the calcium salt was not employed.

Calcium and sodium

The relation between calcium and sodium has been considerably studied. Kearney and Cameron (1902), employing alfalfa and lupine, found that the greatest endurable concentration of sodium chlorid is .02 mol., while in the presence of calcium chlorid the amount of the sodium may be raised to .2 mol.

Attention has already been called to Loeb's (1905 a) experiments with the fish *Fundulus.* This animal cannot live in pure solutions of sodium chlorid isotonic with sea water. It can live for a long time in distilled water or in a mixture of NaCl, KCl, and $CaCl_2$ in the same proportions in which these salts are present in sea water. This is taken to show that the Ca and K ions are required only to overcome the poisonous effects of the sodium ion.

From experiments with wheat seedlings, Osterhout (1908) concludes that mutual antagonism exists between calcium and sodium. This is based on data of which the following form a part:

TABLE 9. WHEAT (GROWTH DURING THIRTY DAYS). QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF .12 M SOLUTIONS

Culture solution	Aggregate length of roots per plant (millimeters)
$CaCl_2$	85
100 $CaCl_2$ 100 NaCl	198
5 $CaCl_2$ 100 NaCl	440
1 $CaCl_2$ 100 NaCl	300
NaCl	55
Distilled water	725

Osterhout states also that this relation is further established as a result of experiments with algæ, liverworts, and *Equisetum.*

The results cited are unusual and inexplicable in view of the greater root growth in distilled water than in the solutions of the combined salts, which result the writer is wholly unable to duplicate. It is noteworthy that the most favorable growth in the mixed solution occurs when the calcium-sodium ratio is 1:20. In any case the protective action of the calcium is apparent, and it is important that so small a quantity of this substance is here required.

Aside from the discrepancy just cited, the writer's results with field peas are fairly comparable with those already mentioned. The data are presented in detail in tables 10 and 11. The sodium salt is strongly deleterious. A much stronger solution of sodium chlorid is required to prevent top growth than to prevent further elongation of roots. When N/50 NaCl is employed, slight development of tops occurs but there is no root extension. When seedlings are placed in N/100 NaCl, the development of tops is appreciable and the elongation of roots is very slight.

In a mixed solution N/5000 $CaCl_2$ + N/50 NaCl, the injurious property of the sodium is entirely counteracted. Thus calcium is a powerful antidote for sodium that is present in an injurious concentration. The same beneficial relation exists when the calcium ion is present in much stronger concentration, for example, in N/50 NaCl + N/20 $CaCl_2$. The growth of the seedlings is greater than in the pure solutions of calcium. Moreover, the results do not indicate, in general, that the most favorable ratio will involve a relatively small proportion of calcium.

TABLE 10. Experiment with Canada Field Pea. Duration, Twenty-four Days. Data for Ten Plants

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 NaCl+N/20 $CaCl_2$	6.10	4.00	10.10	14.0	14.0
N/50 NaCl+N/500 $CaCl_2$	5.15	3.80	8.95	12.0	14.0
N/50 NaCl+N/1,000 $CaCl_2$	6.20	3.40	9.60	15.0	11.0
N/50 NaCl+N/2,000 $CaCl_2$	6.00	3.45	9.45	15.5	10.5
N/50 NaCl+N/5,000 $CaCl_2$	6.10	3.25	9.35	15.0	10.0
N/100 NaCl+N/1,000 $CaCl_2$	4.70	2.50	7.20	12.5	9.0
N/50 NaCl	Small	0	Small	3.0	0
N/100 NaCl	Small	Inap.*	Small	4.0	Slight
N/20 $CaCl_2$	5.00	2.90	7.90	9.0	12.0
Distilled water	1.82	1.75	3.57	6.0	7.0

*Inappreciable.

TABLE 11. EXPERIMENT WITH CANADA FIELD PEA. DURATION, TWENTY-FOUR DAYS. DATA FOR TEN PLANTS

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/100 NaCl+N/1,000 $CaCl_2$...	5.25	3.90	9.15	18.0	12.0
N/100 NaCl+N/2,000 $CaCl_2$...	6.50	4.60	11.10	25.0	14.0
N/100 NaCl+N/4,000 $CaCl_2$...	4.80	3.70	8.50	17.0	11.0
N/100 NaCl..................	Small	Inap.*	Small	4.0	2.0
N/1,000 $CaCl_2$................	6.20	3.30	9.50	22.0	13.0
N/2,000 $CaCl_2$................	5.10	2.20	7.30	18.0	10.0
N/4,000 $CaCl_2$................	4.80	2.10	6.90	19.0	9.5
Distilled water................	2.20	1.80	4.00	7.0	4.0

*Inappreciable.

Calcium and ammonium

The salts of ammonium are extremely injurious to green plants at relatively dilute concentrations. When wheat seedlings are placed in very dilute solutions of ammonium chlorid the deleterious action is exhibited in a pronounced manner by the inhibition of root growth. Immediate injurious effects on the tops are manifest only when the concentration is considerably increased. (An explanation of this behavior is reserved.) In this connection it is of interest to note that the salts of ammonium are in general considered agents which penetrate the cell more rapidly than do other salts. Experiments conducted by the writer with peas and wheat show conclusively that the deleterious effect of ammonium is greatly reduced by the addition of calcium; yet the calcium relation is apparently not modified by ammonium.

Mazé (1900) states that ammonium salts are poisonous to corn when present in greater amounts than .5 part per 1000 in the following solution:

Distilled water........................	1,000 cubic centimeters
KH_2PO_4...........................	1.0 gram
$CaCO_3$.............................	2.0 grams
$MgSO_4$.............................	0.2 gram
$FeSO_4$.............................	0.1 gram
$MnCl_2$.............................	0.1 gram
$ZnCl_2$.............................	Trace
$K_2Si_2O_5$..........................	Trace

He found the toxicity manifested particularly in the root development. The roots were retarded in their growth, being short and stubby.

TABLE 12. Experiment with Canada Field Pea. Duration, Twenty-four Days. Data for Ten Plants

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
$N/20\ CaCl_2$	5.00	2.90	7.90	8.8	12.0
$N/20\ CaCl_2+N/100\ NH_4Cl$	3.75	2.90	6.65	11.0	12.0
$N/20\ CaCl_2+N/500\ NH_4Cl$	4.25	2.85	7.10	11.2	12.0
$N/20\ CaCl_2+N/1,000\ NH_4Cl$	4.15	2.50	6.65	8.5	12.0
$N/100\ CaCl_2+N/500\ NH_4Cl$	5.72	3.59	9.31	11.5	10.5
$N/1,000\ CaCl_2+N/1,000\ NH_4Cl$	5.26	2.65	7.91	12.5	10.0
$N/5,000\ CaCl_2+N/1,000\ NH_4Cl$	3.55	2.45	6.00	8.0	6.0
$N/30\ NH_4Cl$	Not taken	—	—	2.0	0
$N/500\ NH_4Cl$	1.65	1.80	3.45	6.0	6.0
$N/1,000\ NH_4Cl$	1.72	1.90	3.62	6.5	6.0
$N/5,000\ NH_4Cl$	1.80	2.10	3.90	6.3	6.5
$N/100\ CaCl_2$	5.70	3.20	8.90	10.0	13.0
Distilled water	1.85	1.76	3.61	6.0	7.0

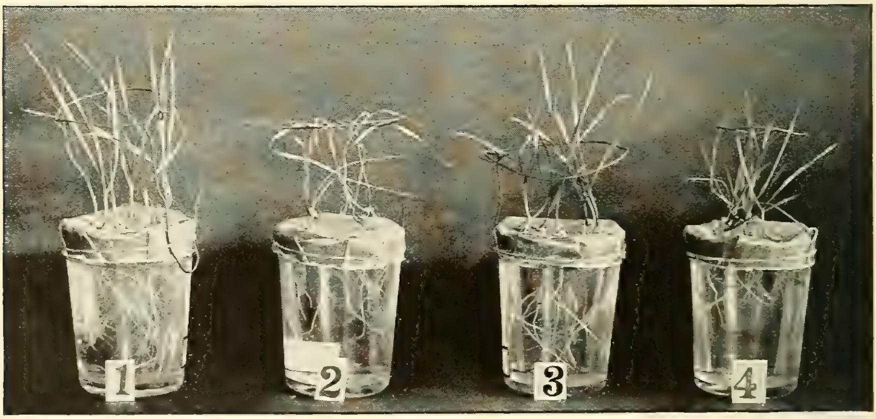

Fig. 4.—*Antidotal relations between calcium and ammonium; distilled water as the solvent*

1. $N/20\ CaCl_2$
2. $N/20\ CaCl_2+N/500\ NH_4Cl$
3. $N/50\ CaCl_2+N/1000\ NH_4Cl$
4. $N/1000\ NH_4Cl$

TABLE 13. EXPERIMENT WITH CANADA FIELD PEA. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution	Green weight of roots (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/100 $CaCl_2$+N/1,000 NH_4Cl	2.00	9.0	8.0
N/100 $CaCl_2$+N/2,000 NH_4Cl	2.00	11.0	10.0
N/100 $CaCl_2$+N/3,000 NH_4Cl	2.54	10.0	10.0
N/100 $CaCl_2$+N/4,000 NH_4Cl	2.03	10.0	9.5
N/30 NH_4Cl	0	2.0	0
N/500 NH_4Cl	Weight not taken	2.0	Slight
N/2,000 NH_4Cl	1.63	4.0	3.0
N/3,000 NH_4Cl	1.67	4.0	3.0
N/4,000 NH_4Cl	1.70	4.0	4.0
N/100 $CaCl_2$	2.85	13.0	8.0
Distilled water	1.80	7.0	5.0

TABLE 14. EXPERIMENT WITH WHEAT. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution	Total green weight of plants (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/100 $CaCl_2$+N/1,000 NH_4Cl	0.95	12.5	12.5
N/100 $CaCl_2$+N/2,000 NH_4Cl	1.20	15.0	15.0
N/100 $CaCl_2$+N/3,000 NH_4Cl	1.40	15.5	12.5
N/100 $CaCl_2$+N/4,000 NH_4Cl	1.15	13.0	10.0
N/2,000 NH_4Cl	1.16	9.0	Very slight
N/3,000 NH_4Cl	1.10	10.0	Very slight
N/4,000 NH_4Cl	1.16	11.0	8.0
N/100 $CaCl_2$	2.88	17.5	27.5
Distilled water	1.71	10.5	17.5

The relations of calcium and ammonium in the complex nutrient solution have also been followed. The nutrient solution was of the same composition as that employed in the first series in which the calcium and magnesium relations were studied.

An examination of the data set forth in Table 15 shows that in this solution N/25 NH_4Cl is very injurious to the tops of seedlings, and

TABLE 15. Experiment with Canada Field Pea. Duration, Twenty-five Days. Data for Ten Plants

Composition of solution (nutrient solution as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/10 $CaCl_2$+N/25 NH_4Cl	1.90	2.60	4.50	6.0	6.0
N/25 $CaCl_2$+N/25 NH_4Cl	5.50	4.52	10.02	11.0	12.0
N/50 $CaCl_2$+N/25 NH_4Cl	4.29	3.90	8.19	10.0	12.0
N/100 $CaCl_2$+N/25 NH_4Cl	4.91	4.95	9.86	9.0	11.0
N/500 $CaCl_2$+N/25 NH_4Cl	3.10	4.15	7.25	7.0	12.0
N/25 $CaCl_2$+N/500 NH_4Cl	11.95	7.80	19.75	18.0	13.0
N/25 $CaCl_2$+N/100 NH_4Cl	11.08	5.80	16.88	17.0	13.0
N/10 $CaCl_2$+N/50 NH_4Cl	6.81	4.31	11.12	11.0	8.0
N/50 $CaCl_2$+N/50 NH_4Cl	11.11	6.20	17.31	15.0	14.0
N/100 $CaCl_2$+N/50 NH_4Cl	10.35	5.70	16.05	14.0	12.0
N/500 $CaCl_2$+N/50 NH_4Cl	9.45	4.60	14.05	14.0	12.0
N/1,000 $CaCl_2$+N/50 NH_4Cl	8.45	4.38	12.83	12.0	12.0
N/25 NH_4Cl	3.50	3.80	7.30	7.0	12.0
N/50 NH_4Cl	9.75	4.85	14.60	11.0	11.0
N/100 NH_4Cl	8.80	4.40	13.20	12.0	12.5
N/500 NH_4Cl	9.60	6.56	16.16	17.0	11.0
Nutrient solution	10.40	5.20	15.60	13.0	10.0

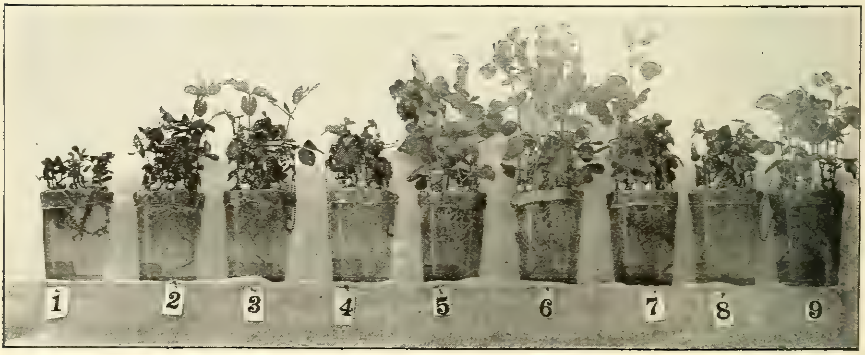

Fig. 5.— *Antidotal relations between calcium and ammonium; nutrient solution as the solvent*

1. N/10 $CaCl_2$+N/25 NH_4Cl
2. N/25 $CaCl_2$+N/25 NH_4Cl
3. N/100 $CaCl_2$+N/25 NH_4Cl
4. N/500 $CaCl_2$+N/25 NH_4Cl
5. N/10 $CaCl_2$
6. N/25 $CaCl_2$
7. N/500 $CaCl_2$
8. N/25 NH_4Cl
9. Nutrient solution

to a somewhat less extent to the roots. Although slight growth results in the above cultures, death of tops soon occurs. N/50 NH_4Cl is only slightly toxic. The addition of either N/25, N/50, or N/100 $CaCl_2$ reduces the deleterious action of N/25 or N/50 NH_4Cl; whereas N/10 $CaCl_2$ increases the total injury, possibly due, however, to the general increase in concentration.

Sodium and ammonium

Mutual antagonism may be shown to exist between sodium and ammonium ions, wheat seedlings being employed as indicators. The writer's experiments show that death of the seedlings soon results when they are placed in a solution of N/50 NaCl. A solution of N/2000 NH_4Cl is poisonous to the seedlings. The toxicity of the ammonium ions of either N/100, N/500, or N/1000 NH_4Cl is greatly reduced by N/50 NaCl. Solutions of either N/500, N/1000, or N/2000 NaCl appreciably antidote N/1000 NH_4Cl. The toxicity of N/50 NaCl is clearly antidoted by NH_4Cl. The following data from the writer's experiments support these statements:

TABLE 16. EXPERIMENT WITH WHEAT. DURATION, TWENTY-ONE DAYS. DATA FOR EIGHT PLANTS

Composition of solution	Average length of tops (centimeters)	Average length of roots (centimeters)	Total green weight (grams)	Color of tops
N/50 NaCl+N/100 NH_4Cl	8	6	.50	Poor
N/50 NaCl+N/500 NH_4Cl	11	7	.95	Poor
N/50 NaCl+N/1,000 NH_4Cl	11	8	.90	Poor
N/500 NaCl+N/1,000 NH_4Cl	12	6	.75	Poor
N/1,000 NaCl+N/1,000 NH_4Cl	11	6	.72	Poor
N/2,000 NaCl+N/1,000 NH_4Cl	11	6	.69	Poor
N/50 NaCl	Slight growth	Slight growth	Weight not taken	Poor
N/500 NaCl	11	———	.86	Poor
N/2,000 NH_4Cl	4	4	.45	Poor
Distilled water	8	Slight growth	.95	Poor

Calcium and barium

Barium in soils.— Considerable interest was attached to the experiments with barium, since in recent times the occurrence and distribution of barium in soils and in plants has received special attention. According to the results obtained by Failyer (1910), barium is a widely disseminated element. It is present in most soils of the United States, the larger quantities occurring in soils derived from masses carrying barite deposits. The original source in all cases seems to be the feldspars of the igneous rocks, the masses carrying barite being intermediate in formation. Barium may be expected to be present in small amounts in soil water. Crawford (1908) has noted its presence in various plants and has related its occurrence to the loco disease.

Toxicity of barium.—Although closely related to calcium the barium ion is extremely toxic in pure solution, and, since it is widely distributed in soils and is present in many kinds of plants, it is desirable to know definitely the effects on the barium ion of the calcium ion with respect to plant growth.

Relatively little work seems to have been recorded respecting the relations of barium. Through some experiments designed to determine the extent to which this element may replace calcium in the growth of plants, Suzuki (1910) found that, although barium is poisonous to plants at relatively dilute concentrations, this poisonous action can be lessened considerably by the presence of calcium salts.

The data obtained by the writer as a result of experiments with Canada field peas show clearly that barium is extremely poisonous to plants and may be rendered innocuous by calcium. Moreover, when barium and calcium ions are present in proper concentrations, the growth of pea seedlings may be slightly more extensive than in solutions of calcium alone.

TABLE 17. Experiment with Canada Field Pea. Duration, Thirty Days. Data for Ten Plants

Composition of solution	Green weight of roots (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/100 $CaCl_2$+N/2,000 $BaCl_2$	2.30	8.00	9.0
N/100 $CaCl_2$+N/4,000 $BaCl_2$	2.51	10.50	9.5
N/100 $CaCl_2$+N/8,000 $BaCl_2$	2.40	9.50	8.5
N/100 $CaCl_2$+N/10,000 $BaCl_2$	2.50	7.50	9.5
N/1,000 $BaCl_2$	0	0.25	0
N/4,000 $BaCl_2$	Weight not taken	4.00	Slight
N/8,000 $BaCl_2$	2.40	6.00	8.0
N/100 $CaCl_2$	2.65	8.50	9.5
Distilled water	1.43	7.00	6.0

TABLE 18. Experiment with Canada Field Pea. Duration, Twenty-four Days. Data for Ten Plants

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/20 $CaCl_2$+N/250 $BaCl_2$	5.50	3.90	9.40	10.0	8
N/20 $CaCl_2$+N/500 $BaCl_2$	5.45	4.15	9.60	9.5	11
N/20 $CaCl_2$+N/1,000 $BaCl_2$	5.65	3.65	9.30	15.0	9
N/20 $CaCl_2$+N/5,000 $BaCl_2$	4.55	3.70	8.25	9.0	11
N/100 $CaCl_2$+N/10,000 $BaCl_2$	5.25	3.52	8.77	10.5	12
N/1,000 $BaCl_2$	Weight not taken	0	Small	1.0	0
N/5,000 $BaCl_2$	0.82	Weight not taken	——	3.0	Slight
N/10,000 $BaCl_2$	1.82	1.90	3.72	6.5	7
N/20 $CaCl_2$	5.00	2.90	7.90	9.0	12
N/100 $CaCl_2$	5.70	3.20	8.90	10.0	13
Distilled water	1.70	1.75	3.45	6.0	6
N/10 $CaCl_2$	4.00	4.60	8.60	7.0	12
N/10 $CaCl_2$+N/1,000 $BaCl_2$	4.30	5.56	9.86	10.0	12
N/10 $CaCl_2$+N/4,000 $BaCl_2$	4.76	6.50	11.26	9.0	14
Distilled water	1.65	1.82	3.47	5.0	6

Magnesium and barium

With Canada field peas it was found that magnesium and barium antidote each other. The action is not great, however. Furthermore, as exhibited by the data, this antitoxic action is more evident when growth of roots is considered alone than when growth of both roots and tops is considered.

In this series N/800 $MgCl_2$ is approximately the lethal concentration, and in a solution of N/4000 $BaCl_2$ the root growth is slightly less than in distilled water. When magnesium and barium are offered together as N/800 $MgCl_2$ and N/4000 $BaCl_2$, the development is much greater than in distilled water. When a stronger solution of barium is combined with magnesium the solution is toxic. On the other hand, when N/800 $MgCl_2$ and N/6000 $BaCl_2$ are mixed in solution, the poisonous action of the magnesium is not appreciably reduced.

TABLE 19. Experiment with Canada Field Pea. Duration, Thirty Days. Data for Ten Plants

Composition of solution	Green weight of roots (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/800 $MgCl_2$+N/2,000 $BaCl_2$	0	3.0	0
N/800 $MgCl_2$+N/4,000 $BaCl_2$	2.40	8.0	8.0
N/800 $MgCl_2$+N/6,000 $BaCl_2$	1.85	7.0	7.0
N/800 $MgCl_2$	1.35	6.0	3.5
N/2,000 $BaCl_2$	0	3.0	Slight
N/4,000 $BaCl_2$	1.22	6.0	3.0
Distilled water	1.42	7.0	6.0

Potassium and barium

In order to obtain more extensive data with respect to potassium and barium ions, cultures were made up in which these cations were present alone and other cultures in which they were together in solution. The solvent employed was N/1000 $CaCl_2$. It was previously determined that N/1000 $CaCl_2$ is wholly ineffective in preventing the toxicity of barium at concentrations here employed. Osterhout (1907), discussing results obtained from experiments with wheat, states: "If we place grains of wheat in NaCl (or KCl) .05 M very little growth occurs. The addition of a very small quantity of barium (100 c.c. NaCl+1 c.c. $BaCl_2$ .05 M or 100 c.c. KCl .05 M+1 c.c. $BaCl_2$ .05 M) at once produces a splendid growth."

Toxic strengths of potassium and lethal concentrations of barium were employed in the experiments. It was found that when these ions are present together in the weak solution of $CaCl_2$ mentioned, mutual antagonism results. It is to be noted that N/25 KCl is highly injurious to seedlings and N/500 $BaCl_2$ prevents growth. Appreciable top growth takes place and the toxicity toward the roots is reduced, in cultures composed of N/25 KCl+N/500 $BaCl_2$. Similar results are obtained when N/25 KCl is present in solution with either N/1000 or N/2000 $BaCl_2$. In no combination is the top development of the plants so extensive as in those grown in the control, N/1000 $CaCl_2$.

TABLE 20. EXPERIMENT WITH CANADA FIELD PEA. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution ($N/1000$ $CaCl_2$ as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
$N/25$ KCl	Too dry to weigh	3.75	——	3.0	5.0
$N/25$ KCl+$N/500$ $BaCl_2$	1.75	5.37	7.12	5.0	10.0
$N/25$ KCl+$N/1{,}000$ $BaCl_2$	3.15	5.77	8.92	6.0	12.0
$N/25$ KCl+$N/2{,}000$ $BaCl_2$	2.35	4.85	7.20	3.5	7.0
$N/100$ KCl+$N/1{,}000$ $BaCl_2$	2.55	2.68	5.23	6.0	10.0
$N/1{,}000$ KCl+$N/1{,}000$ $BaCl_2$	2.10	2.50	4.60	5.0	8.0
$N/500$ $BaCl_2$	0	0	0	0	0
$N/1{,}000$ $BaCl_2$	Weight not taken	Slight	Slight	3.0	Slight
$N/1{,}000$ $CaCl_2$	5.20	3.46	8.66	8.0	10.0

Calcium, potassium, sodium, or magnesium, and strontium

Suzuki (1910), while endeavoring to determine whether or not strontium can replace calcium in plants, called attention to the toxicity of this element. Osterhout (1907) has indicated that strontium has a protective action with respect to magnesium. He says: "In a solution of .05 M $MgCl_2$ or $MgSO_4$ or $Mg(NO_3)_2$ grains of wheat make scarcely any growth. But if to a solution of 100 c.c. $MgCl_2$ .05 M we add 20 c.c. $SrCl_2$ a very fine growth is observed."

The writer finds from experiments with pea and wheat seedlings that calcium, potassium, sodium, and magnesium each reduces the deleterious action of strontium, and that when strontium is present in solution with either of these the growth is greater than in pure solutions of these substances, as shown in the following tables. It will be noted that the growth in solutions in which nitrates were employed greatly exceeds that when chlorids are used. In the different tables, therefore, the data are not comparable.

Calcium and strontium.— From tables 21 and 22 it is evident that calcium and strontium have a mutually protective action. Calcium is very effective in reducing the harmful properties of strontium. $N/100$ $Sr(NO_3)_2$ is strongly toxic to both tops and roots of seedlings, while $N/500$ $Sr(NO_3)_2$ is injurious to the roots. When solutions of $N/20$ $Ca(NO_3)_2$ and $N/100$ $Ca(NO_3)_2$, respectively, are mixed with the above concentrations of strontium the toxicity is entirely overcome. It is to be noted further that the addition of N/100, N/250, N/500, or N/1000 $Sr(NO_3)_2$ to N/20 $Ca(NO_3)_2$ results in a greater production of both tops and roots than occurs in N/20 $Ca(NO_3)_2$ alone. The increase in growth cannot be attributed to the addition of the nitrate radical to the calcium solution, inasmuch as similar relations exist between the chlorids of these metals.

The more concentrated solutions of strontium cause an increase in diameter of the roots of pea seedlings. Slight elongation takes place and no lateral roots are formed. As indicated in Table 21, less concentrated solutions are required to injure the roots than to produce injury to the tops.

TABLE 21. Experiment with Canada Field Pea. Duration, Twenty-four Days. Data for Ten Plants

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/20 $Ca(NO_3)_2$+N/100 $Sr(NO_3)_2$..	7.80	4.40	12.20	23.0	13.0
N/20 $Ca(NO_3)_2$+N/250 $Sr(NO_3)_2$..	7.20	4.20	11.40	22.5	12.5
N/20 $Ca(NO_3)_2$+N/500 $Sr(NO_3)_2$..	7.25	4.27	11.52	23.5	14.0
N/20 $Ca(NO_3)_2$+N/1,000 $Sr(NO_3)_2$.	9.20	4.70	13.90	24.0	11.0
N/100 $Ca(NO_3)_2$+N/250 $Sr(NO_3)_2$.	7.85	3.70	11.55	19.0	9.0
N/100 $Ca(NO_3)_2$+N/500 $Sr(NO_3)_2$.	7.35	3.45	10.80	18.5	8.0
N/20 $Ca(NO_3)_2$..................	7.30	3.20	10.50	16.0	11.5
N/100 $Ca(NO_3)_2$.................	7.95	3.70	11.65	16.0	7.9
N/50 $Sr(NO_3)_2$...................	Slight	0	—	2.0	Slight
N/100 $Sr(NO_3)_2$..................	1.60	1.20	2.80	5.5	4.0
N/500 $Sr(NO_3)_2$..................	3.20	1.35	4.55	8.5	3.5
N/1,000 $Sr(NO_3)_2$................	2.40	1.50	3.90	8.0	6.0
Distilled water...................	1.75	1.70	3.45	6.0	6.5

TABLE 22. Experiment with Canada Field Pea. Duration, Thirty Days. Data for Ten Plants

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/10 $CaCl_2$....................	4.10	4.60	8.70	7.0	12.0
N/10 $CaCl_2$+N/500 $SrCl_2$......	4.30	6.10	10.40	9.0	14.0
N/500 $SrCl_2$.................	1.45	1.24	2.69	6.0	5.5
Distilled water................	1.65	1.82	3.47	5.0	6.0

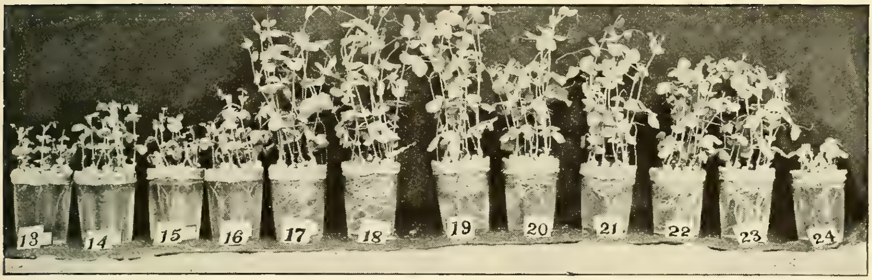

FIG. 6.—*Antidotal relations between calcium and strontium; distilled water as the solvent*

13. N/100 $Sr(NO_3)_2$
14. N/500 $Sr(NO_3)_2$
15. N/1000 $Sr(NO_3)_2$
16. N/5000 $Sr(NO_3)_2$
17. N/20 $CaCl_2$ + N/100 $Sr(NO_3)_2$
18. N/20 $CaCl_2$ + N/500 $Sr(NO_3)_2$
19. N/20 $CaCl_2$ + N/1000 $Sr(NO_3)_2$
20. N/100 $CaCl_2$ + N/250 $Sr(NO_3)_2$
21. N/100 $CaCl_2$ + N/500 $Sr(NO_3)_2$
22. N/20 $CaCl_2$
23. N/100 $CaCl_2$
24. Distilled water

Potassium and strontium.—Both potassium and strontium are strongly toxic to the roots of wheat seedlings. It is to be noted in Table 23 that the root development is slight in solutions of either N/50 KCl or N/2000 $SrCl_2$. It is shown further that the top growth of the wheat in these solutions is greater than that in distilled water. The deleterious action is counteracted when these salts are mixed in distilled water. In a solution containing N/50 KCl + N/2000 $SrCl_2$ the development of both tops and roots of the seedlings is greater than those grown in individual solutions of these salts. When N/1000 KCl and N/1000 $SrCl_2$ are combined, the poisonous action of the strontium is but slightly reduced.

TABLE 23. EXPERIMENT WITH WHEAT. DURATION, TWENTY-FIVE DAYS. DATA FOR TEN PLANTS

Composition of solution	Total green weight of plants (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 KCl + N/100 $SrCl_2$	0.762	8.0	9.0
N/50 KCl + N/500 $SrCl_2$	1.250	10.0	9.0
N/50 KCl + N/2,000 $SrCl_2$	1.500	10.0	10.0
N/1,000 KCl + N/1,000 $SrCl_2$	1.000	8.0	2.0
N/2,000 $SrCl_2$	0.750	7.5	1.5
N/20 KCl	0	0	0
N/50 KCl	0.810	8.0	1.5
Distilled water	0.680	7.0	6.0

Sodium and strontium.—With these bases also, as with potassium and strontium, is shown a clear case of mutual antagonism. By combining a solution of sodium with one of strontium, each of which, when alone in solution, is detrimental to wheat plants, no detrimental action results. Sodium is not so effective as potassium in overcoming the harmful properties of strontium. For example, an N/500 NaCl solution does not reduce the toxicity of N/1000 $SrCl_2$ to any greater extent than does a solution of N/1000 KCl. A comparison of the data in Table 23 with those in Table 24 discloses the fact that the growth of wheat seedlings is less when placed in cultures containing sodium and strontium than when placed in solutions of similar composition of potassium and strontium.

TABLE 24. Experiment with Wheat. Duration, Twenty-five days. Data for Ten Plants

Composition of solution	Total green weight of plants (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 NaCl	0.51	4.0	0
N/50 NaCl+N/100 $SrCl_2$	0.71	6.0	5.0
N/50 NaCl+N/500 $SrCl_2$	1.05	8.5	9.0
N/50 NaCl+N/2,000 $SrCl_2$	1.10	9.0	8.0
N/500 NaCl+N/1,000 $SrCl_2$	1.10	7.0	2.0
N/2,000 $SrCl_2$	0.75	7.5	1.5
Distilled water	0.68	7.0	6.0

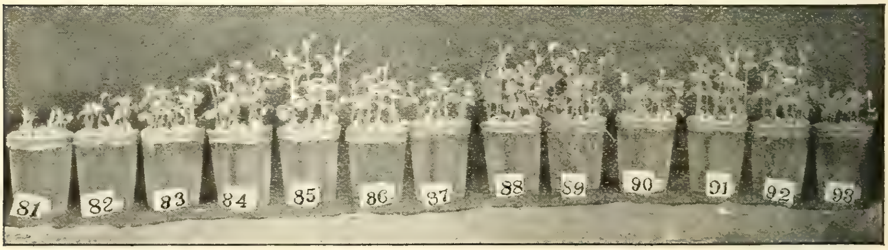

Fig. 7.—*Antidotal relations between sodium and strontium; distilled water as the solvent*

81. N/20 $NaNO_3$
82. N/50 $NaNO_3$
83. N/100 $NaNO_3$
84. N/50 $NaNO_3$+N/500 $Sr(NO_3)_2$
85. N/50 $NaNO_3$+N/1000 $Sr(NO_3)_2$
86. N/50 $NaNO_3$+N/3000 $Sr(NO_3)_2$
87. N/500 $NaNO_3$+N/500 $Sr(NO_3)_2$
88. N/500 $NaNO_3$+N/1000 $Sr(NO_3)_2$
89. N/500 $NaNO_3$+N/3000 $Sr(NO_3)_2$
90. N/100 $Sr(NO_3)_2$
91. N/500 $Sr(NO_3)_2$
92. N/5000 $Sr(NO_3)_2$
93. Distilled water

Magnesium and strontium.— It has been seen that magnesium and strontium are toxic at comparatively weak concentrations. However, when these bases are combined in the proper proportions and concentrations this toxicity is mutually counteracted.

Inasmuch as Canada field pea seedlings elongate but little in the absence of calcium, a trace of this compound — N/1000 $CaCl_2$ — was added to each of the cultures in the experiments noted below. With the increase in growth obtained by the addition of calcium the antidoting action of the bases magnesium and strontium is more obvious.

TABLE 25. EXPERIMENT WITH CANADA FIELD PEA. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution (N/1000 $CaCl_2$ as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/200 $MgCl_2$+N/100 $SrCl_2$....	1.35	3.05	4.40	5	9
N/200 $MgCl_2$+N/500 $SrCl_2$....	2.76	3.10	5.86	7	9
N/200 $MgCl_2$+N/1,000 $SrCl_2$...	3.75	3.70	7.45	7	10
N/500 $MgCl_2$+N/500 $SrCl_2$....	3.50	3.20	6.70	8	8
N/1,000 $MgCl_2$+N/500 $SrCl_2$...	3.20	3.06	6.26	8	9
N/100 $SrCl_2$..................	1.00	3.20	4.20	3	8
N/500 $SrCl_2$..................	3.55	2.10	5.65	7	9
N/200 $MgCl_2$..................	1.45	2.14	3.59	4	7
N/1,000 $CaCl_2$................	5.01	3.35	8.36	8	10

The writer's experiments demonstrate a definite antagonistic action between magnesium and strontium. The toxicity is not entirely overcome, since the growth in the mixed solutions is not so extensive as in the control, N/1000 $CaCl_2$. Mutual antagonism exists when these bases are present in diverse ratios; for example, it occurs in solutions made up as follows: N/200 $MgCl_2$+N/100 $SrCl_2$ and N/200 $MgCl_2$+N/1000 $SrCl_2$. The greater growth occurs in the latter solution.

Sodium and potassium

Osterhout (1909), in working with wheat, observed that mutual antagonism exists between sodium and potassium. The data presented below are taken from one of his tables:

TABLE 26. WHEAT (GROWTH DURING THIRTY DAYS). QUANTITIES GIVEN ARE CUBIC CENTIMETERS OF .12 M SOLUTIONS

Culture solution	Aggregate length of roots per plant (millimeters)
NaCl	55
100 NaCl 10 KCl	115
100 NaCl 35 KCl	130
35 NaCl 100 KCl	80
KCl	65
Distilled water	725

Hansteen (1909) found only slight antagonism between sodium and potassium in distilled water. Wheat was used as the indicator.

The harmful influences of potassium and sodium are reduced when these bases are combined even in widely different ratios. From the experiments with pea seedlings it is seen that a solution of N/500 NaCl very slightly reduces the toxicity of N/50 KCl. The minimum amount of potassium that may antidote a given amount of sodium was not determined, but relatively dilute solutions reduce the poisonous action of comparatively concentrated solutions of sodium. The solutions used and the data obtained are given in the following table:

TABLE 27. EXPERIMENT WITH CANADA FIELD PEA. DURATION, TWENTY-FOUR DAYS. DATA FOR TEN PLANTS

Composition of solution	Green weight of roots (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 NaCl+N/50 KCl	2.56	6.00	4.00
N/50 NaCl+N/100 KCl	3.30	8.00	7.00
N/50 NaCl+N/500 KCl	4.40	11.00	11.00
N/100 NaCl+N/50 KCl	2.25	6.50	4.00
N/500 NaCl+N/50 KCl	1.60	5.00	Very slight
N/50 KCl	——	0.75	0
N/100 KCl	1.70	5.00	4.00
N/500 KCl	1.80	5.50	4.50
N/50 NaCl	0	Slight	0
N/100 NaCl	Slight	4.00	2.00
N/500 NaCl	2.60	7.00	7.00
Distilled water	1.74	6.00	6.50

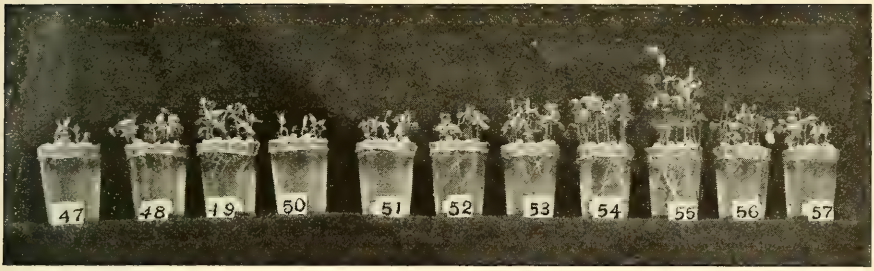

FIG. 8.— *Antidotal relations between sodium and potassium; distilled water as the solvent*

47. N/50 KCl
48. N/100 KCl
49. N/500 KCl
50. N/50 NaCl
51. N/100 NaCl
52. Distilled water
53. N/50 NaCl + N/50 KCl
54. N/50 NaCl + N/100 KCl
55. N/50 NaCl + N/500 KCl
56. N/100 NaCl + N/50 KCl
57. N/500 NaCl + N/50 KCl

A comparison of the data in Table 27 with those in tables 7 and 10 reveals the fact that calcium is far more effective in overcoming the harmful action of either sodium or potassium than is any combination of the latter. Both the top and the root growth are greater when either of these bases is combined with calcium than when the two bases are combined together.

At the close of the experiment the color of the tops in all solutions was very poor. This was no doubt due to the absence of calcium from the cultures.

THE INFLUENCE OF AGE OF SEEDLINGS ON TOXIC AND ANTITOXIC RELATIONS

The influence of age of seedlings on the toxicity of barium, sodium, strontium, and ammonium, and the antidotal relations between calcium and each of these ions, have been studied. Pea seedlings were grown for ten days in a full nutrient solution. They were then removed, carefully rinsed with distilled water, and placed in pure solutions of $CaCl_2$ and in solutions of $CaCl_2$ mixed with each of the above salts. Twenty days later the experiments were discontinued. The data in the following paragraphs are obtained from seedlings thus treated.

Calcium and barium

Additional antidotal relations of calcium and barium with respect to pea seedlings are summarized in the following table:

TABLE 28. Experiment with Canada Field Pea. Duration, Thirty Days. Data for Ten Plants

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/10 $CaCl_2$+N/100 $BaCl_2$...	5.20	4.20	9.40	13.0	8.0
N/10 $CaCl_2$+N/500 $BaCl_2$...	5.80	4.70	10.50	11.0	9.0
N/10 $CaCl_2$+N/1,000 $BaCl_2$.	7.40	5.20	12.60	13.0	8.5
N/500 $BaCl_2$...............	Dead	Slight	——	Very slight growth	Very slight growth
N/1,000 $BaCl_2$.............	4.20	2.85	7.05	17.0	7.0
Distilled water.............	5.30	3.45	8.75	10.0	6.0

A comparison of the data presented in tables 17 and 28 reveals the fact that older seedlings are far more resistant to barium ions. It may be cited, for example, that N/4000 $BaCl_2$ prevents further elongation of the roots of young seedlings, whereas N/500 is required to kill older seedlings. The greater resistance may be due to mass relations; that is, the salts taken up from the nutrient solution may prevent the entrance of barium ions from the weaker solutions. Again, the more nearly complete migration of the storage products from the seed to the root may react in like manner. Furthermore, the permeability of the cell membrane may be altered and thus the entrance of the toxic ions may be retarded. The first postulation is unlikely, as the writer has found from several sets of experiments that seedlings which have been grown for ten days in distilled water are apparently no more susceptible to toxic ions than are those grown for the same length of time in full nutrient solution.

Stronger solutions of barium are required to exert toxic action toward older seedlings; but in this case also calcium is effective in preventing the injurious action. When N/10 $CaCl_2$ is present in solution with either N/100, N/500, or N/1000 $BaCl_2$, no injury results.

Calcium and sodium

The results obtained from further studies of the relations between calcium and sodium are similar to those just discussed. Older seedlings are more tolerant of sodium ions than are younger ones. Seedlings taken from the germinating pan and placed directly in N/100 NaCl soon perish. On the other hand, those that are grown for ten days in a full nutrient solution withstand any solutions weaker than N/20. Calcium chlorid of N/10 concentration prevents injury from N/20 NaCl. The data obtained appear in Table 29:

TABLE 29. EXPERIMENT WITH PEA SEEDLINGS. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/10 $CaCl_2$ +N/20 NaCl	6.85	5.65	15.0	9.0
N/10 $CaCl_2$ +N/100 NaCl	7.14	6.37	13.0	8.0
N/10 $CaCl_2$ +N/500 NaCl	8.18	7.37	15.0	8.0
N/10 NaCl	No growth	Weight not taken	——	——
N/20 NaCl	Dead	Weight not taken	Slight growth	——
N/100 NaCl	5.00	4.00	9.5	7.5
N/500 NaCl	4.60	4.25	10.0	8.0
N/10 $CaCl_2$	7.90	6.00	17.0	7.0
Distilled water	5.30	3.45	10.0	6.0
Nutrient solution	12.40	8.25	15.0	10.0

Calcium and strontium

Older seedlings are also more resistant to strontium ions than are younger seedlings. Calcium is effective in preventing this deleterious action, since no injury results in solutions composed of N/100 $CaCl_2$+N/50 $SrCl_2$. The essential data are set forth in the following table:

TABLE 30. EXPERIMENT WITH CANADA FIELD PEA. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
$N/10\ CaCl_2 + N/50\ SrCl_2$	7.50	7.30	14.80	15	9
$N/10\ CaCl_2 + N/100\ SrCl_2$	9.00	5.90	14.90	15	8
$N/10\ CaCl_2 + N/500\ SrCl_2$	7.64	5.75	13.39	16	7
$N/100\ CaCl_2 + N/50\ SrCl_2$	5.36	4.45	9.81	12	8
$N/50\ SrCl_2$	Dead, weight not given	4.45	——	6	11
$N/100\ SrCl_2$	Dead, weight not given	5.60	——	7	11
$N/500\ SrCl_2$	5.50	4.27	9.77	11	8
$N/10\ CaCl_2$	9.75	6.00	15.75	17	7
Distilled water	5.40	3.55	8.95	10	6
Full nutrient solution	10.40	8.65	19.05	15	10

Calcium and ammonium

Seedlings that have been grown for ten days in full nutrient solutions are also more resistant to ammonium than are those taken from the germinating pan and placed directly in the solutions. $N/100\ NH_4Cl$ is the weakest solution that is toxic in the former case and N/3000 to N/4000 in the latter. The toxicity of either N/25 or $N/100\ NH_4Cl$ is reduced by the presence of $N/10\ CaCl_2$. The results obtained appear in the following table:

TABLE 31. EXPERIMENT WITH CANADA FIELD PEA. DURATION, THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
$N/10\ CaCl_2 + N/25\ NH_4Cl$	Weight not taken	13	Dead after ten days
$N/10\ CaCl_2 + N/100\ NH_4Cl$	12.21	18	8
$N/10\ CaCl_2 + N/500\ NH_4Cl$	16.35	22	7
$N/10\ CaCl_2$	18.85	24	11
$N/25\ NH_4Cl$	——	Dead	——
$N/100\ NH_4Cl$	13.35	12	7
Distilled water	11.95	14	8

DISCUSSION

The reports of favorable results from the application of lime to various types of soil require only the briefest consideration here, since in this report only antagonistic phenomena are referred to. In soil studies the factors and relations are complex, and it is often difficult, if not impossible, to evaluate carefully the antagonistic features. Leaving out of consideration the effects of liming on acidity, its relation to nitrogen fixation and to toxic organic constituents of the soil, and the like, there remains the fact that the addition of lime compounds, both alone and in conjunction with other fertilizers, has resulted in marked increases in yield. The injurious action resulting when unbalanced conditions prevail, and the importance of calcium in correcting this action, make it probable that in many cases the beneficial effects obtained from calcium compounds are due to antagonistic relations. In any event the general trend of results indicates that where any single fertilizer has proved injurious it would be well to apply a calcium compound, at least in limited quantity.

It is not proposed to discuss in detail the views regarding the causes of antagonistic action, but some reference has already been made to the "calcium-protein" theory of Loew (1892 a). Mention should likewise be made of some subsequent papers. Loeb (1905 a) developed further the conception of ion protein compounds in explaining certain aspects of antagonistic action. With the development of colloidal chemistry and the discovery of antagonistic action with respect to the action of salts on proteins, new possibilities became apparent. By employing plasmolytic methods Osterhout (1911) finds that the mechanism of antagonistic action may depend largely on the mutual action of the antagonistic salts in preventing each other from entering, but there must also be taken into account their effect on the protoplasm. Later (1912) he confirms this statement. Loeb (1911) concludes that "the rôle of salts in the preservation of life consists in the 'tanning' effect which they have upon the surface films of the cells, whereby these films acquire those physical qualities of durability and comparative impermeability, without which the cell cannot exist." On the other hand, True and Bartlett (1912) submit evidence from electrolytic conductivity determinations indicating that it is not merely the endosmosis of solutes, but likewise the exosmosis of solutes, which is affected by concentration and relation of the salts. The writer has made some experiments which show that the permeability of the cell is altered, the data of which are reserved for future consideration.

SUMMARY

The results of extensive studies of the toxic and the antidotal action of various ions have been presented. A general summary of these results appears below:

Pure solutions of N/500 $MgCl_2$ or $MgSO_4$ prevent further elongation of roots of seedlings. The toxicity of this cation is prevented by calcium in cultures composed of N/20 $CaCl_2$ with N/20 or any greater dilution of $MgCl_2$; and N/1000 $CaCl_2$ is effective with N/200 $MgCl_2$, but not with N/20 $MgCl_2$. N/50 $MgCl_2$ or higher concentrations are toxic in a dilute full nutrient solution. The further addition of $CaCl_2$ counteracts this injurious action.

Mutual antagonism exists for wheat seedlings when N/50 KCl is present in solution with N/100 to N/1000 $MgCl_2$. N/1000 KCl likewise counteracts the toxicity of N/500 $MgCl_2$.

The sodium and magnesium relations are similar to those of potassium and magnesium; however, sodium is not so effective as potassium in antidoting the magnesium ion.

No elongation of roots of pea seedlings takes place in N/20 KCl; but the presence of $CaCl_2$ at a concentration of N/20 to N/500 completely counteracts this deleterious action.

Very dilute solutions of calcium are effective in overcoming the poisonous action of sodium. N/5000 $CaCl_2$, for example, prevents injury by N/50 NaCl, and this ratio is almost as effective as when N/20 $CaCl_2$ is used with N/50 NaCl.

N/4000 NH_4Cl is toxic to pea and wheat seedlings, the elongation of roots being less than in distilled water. The presence of N/5000 $CaCl_2$ in solution with N/1000 NH_4Cl greatly lowers the harmful action of ammonium ions. No injury results to pea seedlings placed in cultures composed of N/20 $CaCl_2$ + N/100 NH_4Cl.

Salts of ammonium in a full nutrient solution are far less toxic than in distilled water. N/100 NH_4Cl is only slightly injurious with the nutrient solution employed, but with N/25 death of seedlings soon results. The further addition of $CaCl_2$ so that its concentration becomes N/25 or N/100 entirely counteracts the toxicity in the one case and decreases the injury in the other.

As contrasted with their independent action, the poisonous action of sodium and ammonium ions is greatly decreased when present together in solution. This holds when N/50 NaCl is combined with N/100 to N/1000 NH_4Cl, and also when N/1000 NH_4Cl is combined with N/100 to N/1000 NaCl.

Barium ions are very toxic, the elongation of roots of pea seedlings in N/4000 $BaCl_2$ being only about two centimeters. These ions are rendered innocuous by calcium, since growth of seedlings is not retarded when placed in culture solutions composed of N/20 $CaCl_2$ + N/250 $BaCl_2$.

Magnesium and potassium ions also are fairly effective antidotes for barium. On the other hand, barium is a powerful antidote for magnesium and potassium. Thus, the toxicity of N/800 $MgCl_2$ is prevented by the presence of N/6000 $BaCl_2$; the deleterious action of N/25 KCl is overcome by N/2000 $BaCl_2$ and N/1000 $CaCl_2$ (N/1000 $CaCl_2$ being the solvent).

Pure solutions of strontium, even when very dilute, retard the growth of seedlings. Calcium, potassium, sodium, and magnesium each reduces the deleterious action of strontium. Strontium, although present in relatively high dilution, is effective in reducing the toxicity of either potassium, sodium, or magnesium ions.

Mutual antagonism results when potassium and sodium ions are present together in solution. Greater development of seedlings takes place in a solution composed of N/500 KCl + N/50 NaCl than in one composed of N/50 KCl + N/500 NaCl, thus showing that potassium is a more effective antidote for sodium than sodium is for potassium.

The results obtained from the studies of the influence of age of seedlings on toxic and antitoxic action show in general that older seedlings are far more resistant than younger ones to the toxicity of either barium, sodium, strontium, or ammonium ions, and furthermore that calcium is effective in counteracting the toxicity of these ions.

CONCLUSIONS

The chief conclusions to be derived from these experiments are as follows:

1. Each of the following (in the order given) is poisonous to seedlings: Ba, Sr, NH_3, Mg, Na, K.

2. Although mutual antagonism results when cations are present together in solution as follows:

Mg and Sr
K and Sr
Na and Sr
Na and K
Na and NH_3
K and Ba
Mg and Ba

yet calcium is the most effective of any of the substances studied, in preventing toxic action.

3. Protective action is not confined to the so-called essential nutrients, since, as indicated above, Na, Sr, and Ba (the nonessential ions among those here discussed) possess this property.

4. The favorable results obtained from the application of lime to many types of soils is doubtless due in part to the antidotal relations.

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THE ACTION OF CERTAIN NUTRIENT AND NON-NUTRIENT BASES ON PLANT GROWTH — II

THE TOXICITY OF MANGANESE AND THE ANTIDOTAL RELATIONS BETWEEN THIS AND VARIOUS OTHER CATIONS WITH RESPECT TO GREEN PLANTS *

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Manganese is widely distributed in nature. It occurs in various soil types, in some instances in sufficient amount to be injurious to certain crops. It is likewise present in a variety of plants. The rôle of manganese in plant nutrition is not clearly understood, but the metal is considered to be of importance in connection with the oxidizing enzymes. The salts of manganese have been shown to have a stimulating action on plants when applied to soils in small quantities; but in larger quantities the effect is of course detrimental.

The studies that are included in this report consider two chief relations: (1) the toxicity of manganese in distilled water, in full nutrient solution, and in soil cultures; and (2) the antidotal relations that exist between manganese and each of the bases calcium, potassium, sodium, and magnesium.

OCCURRENCE OF MANGANESE IN SOILS

As stated above, manganese occurs in various soil types. Fertile soils as a rule contain less than one per cent of this element reckoned as oxid. The following table from Wolff (1870–1880) is cited in order to indicate in general the amounts of manganese and iron found in soils:

Kind of soil	Percentage of Mn_3O_4	Percentage of Fe_2O_3
Loam	.135	2.096
Clay	.180	3.173
Sand	.080	1.039
Humus	.042	0.406

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* Laboratory of Plant Physiology, Cornell University, Contribution No. 10.

Leclerc (1872) found small amounts of this element in the various soils that he studied, and recent work has shown that some types of soil contain unusually large quantities. The data for soils from certain sections of Hawaii are particularly significant, as the following table from Kelley (1908), for black soils of the Wahiawa district, indicates:

	Percentage
Volatile matter	17.70
Mn_3O_4	9.74
Fe_2O_3	19.65
Al_2O_3	15.50
K_2O	0.83
CaO	1.39
P_2O_5	0.21

OCCURRENCE OF MANGANESE IN PLANTS

The earliest report of the existence of manganese in plants which the writer has been able to obtain is that of Heropath, which Rousset (1909) cites. Heropath found this element in the ash of radish, beet, and carrot. Liebig (1851) reported the presence of manganese in tea. Leclerc (1872) concluded from his analyses that manganese occurs in most species of plants, the percentage being rather high in forest trees. Wolff (1870–1880) also reports analyses which show that manganese is present in the wood, bark, and leaves of various forest trees. Maumené (1884) found that no trace of manganese occurs in the parenchymatous tissue of cabbage leaves, but the veins contain relatively large quantities.

The analyses of Pichard (1898) likewise reveal a wide distribution of manganese in seed plants. The leaves, actively growing parts, and reproductive organs contain the greatest amount.

THE RÔLE OF MANGANESE

The rôle of manganese in plant nutrition is not fully understood, but the researches of Bertrand (1897) established the fact that it is important in the action of the oxidases. Bertrand analyzed the ash of the oxidase-containing material obtained from different samples of laccase, and found the percentage of manganese to be rather high. He studied the oxidizing power in these samples toward hydroquinone, showing that this power varied with the amount of manganese present. He then extracted the so-called laccase from lucern, at the time of flowering, and found the amount of manganese present to be very slight. The samples did not oxidize hydroquinone. When small amounts of manganese were added oxidation took place. Bertrand's results with the lucern extract are as follows:

Experiment	Material tested	O_2 absorbed (cubic centimeters)
1	Check, manganese alone	0.3
2	Laccase of lucern	0.2
3	Laccase of lucern plus manganese	6.3

When the manganese was replaced by other metals, such as Fe, Al, Sn, Zn, Cu, Mg, and K, the amount of oxygen absorbed was in no case greater than a few tenths of a cubic centimeter.

Bertrand states further that all manganous salts are capable of oxidizing hydroquinone, pyrogallol, paramidophenol, guaiac, and related compounds. His experiments with the action of manganese salts on these compounds lead to the conception of oxidases as special combinations of manganese. In this conception the manganese would be the real active agent, which functions as an activator and a carrier of oxygen.

Voelcker (1903) observed a deeper green color in the tops of wheat grown in cultures to which small amounts of manganese chlorid were added.

Trillat (1904) found that the addition of manganese to oxidases results in greater activity of these so-called enzymes.

Stoklasa (1911) considers manganese and aluminium to be important in photosynthesis, since they collect mainly in the leaves of plants.

There are numerous reports indicating that manganese may act as a stimulant when applied to soils in small amounts or when present in solution cultures.

Voelcker (1903) reported that in 1902 the addition of various salts of manganese to pots of soil resulted in some cases in a slight increase in yield of wheat over the product in control cultures. Pots of soil were treated with manganese oxids (MnO_2 and Mn_3O_4), chlorid, iodid, carbonate, sulfate, phosphate, and nitrate, at the rate of two hundred pounds per acre. Increase in yield was obtained from cultures fertilized with phosphate, chlorid, sulfate, and the oxid Mn_3O_4. Parallel experiments were run with barley. The oxids caused no increase in yield; all other salts of manganese resulted in a correspondingly greater increase in yield of barley than of wheat.

The studies were continued in 1903 (1904), when again the oxids of manganese applied to pots of soil at the rate of two hundred pounds per acre showed a beneficial influence on wheat; while with barley it was again found that the oxids of manganese do not produce stimulation.

Loew and Honda (1904) transferred seedlings of *Cryptomeria japonica* from the forest to frames of soil. Small doses of $MnSO_4$ were added to the soil, monthly from May to November inclusive, for two years. The manganese acted as a stimulant, causing an appreciable gain in height and total weight of the trees over those grown in the control frames.

Two plots in a paddy field, each of thirty square meters, were treated with a general fertilizer by Aso (1904). To one was applied in addition two hundred grams of $MnCl_2$. Rice plants were transferred to each plot. Taking the yield of the control plot as a unit, the results obtained from the plot treated with manganese were as follows:

Total yield	1.42
Straw	1.48
Full grains	1.36
Full grains (husked)	1.30

The results show an increase in the grain of approximately one third, by an application of manganese. In order to be able to draw definite conclusions from field work, it seems that more plots should be used in the experiments.

Flax was grown by Fukutome (1908) in pots containing eight kilograms of soil. Each pot received a general fertilizer as follows: 16 grams s perphosphate, 10 grams K_2SO_4, 8 grams $(NH_4)_2\ SO_4$, and 8 grams $NaNO_3$. The pots were then treated with special fertilizers. The special fertilizers and the results obtained are indicated in the following table:

Pot	Special fertilizer	Weight of plants, air-dry (grams)
1	Control	10.5
2	.4 gram $MnCl_2+4\ H_2O$	10.7
3	.4 gram $MnCl_2+4\ H_2O$ .4 gram $FeSO_4+7\ H_2O$	12.9
4	.4 gram $FeSO_4+7\ H_2O$	11.6

The manganese and the iron when used alone each causes a small increase in product, but when combined the increase is marked.

Namba (1908) found that the addition of manganese sulfate as a top-dressing to pots of onions containing eight kilograms of soil results in stimulation. The application of greater amounts than .1 gram $MnSO_4$ causes a decrease in stimulating effect.

Loew (1904) found from experiments with pot cultures of soil that manganese stimulates the growth of oats, tobacco, and peas. He found also that the treatment of plots of soil with small quantities of manganese stimulates the growth of radishes, potato, and millet. Manganese is more effective when applied as a top-dressing than when mixed with the soil. It is also better to apply the manganese as several top-dressings than to apply all in one dose.

Bertrand (1905) applied manganese sulfate to a field of oats at the rate of fifty kilograms per hectare. Appreciable stimulation resulted. The gain in the total crop was 22½ per cent. Bertrand considers the action of the manganese to be catalytic.

Labergerie (1908) conducted experiments using manganese as a fertilizer for wheat. His results have been reported as follows:

"In case of wheat on dry sandy soil containing small amounts of lime both the chlorid and the sulphate increased the yield. On a more moist soil the chlorid decreased the yield slightly and the sulphate caused a small increase. In all cases the sulphate was more effective than the chlorid, but both were less effective on wet soil than on dry. . . . . The manganese salts were applied at the rate of 22.27 lbs. of manganese oxid per acre by dissolving in a considerable volume of water and sprinkling on the soil."

Experiments conducted by Stoklasa (1911) show that manganese and aluminium, when present in small quantities in nutrient solution, each stimulates several species of plants. Stoklasa is of the opinion that these elements are of importance in photosynthesis, inasmuch as they collect mainly in the leaves. Further, the presence of these elements in the culture solution results in the formation of larger leaves.

The nutrient solution employed was made up as follows:

K_2SO_4	1.00 gram
$MgCl_2$	0.50 gram
$NaNO_3$	0.50 gram
$FeSO_4$	0.01 gram
$CaSO_4$	0.05 gram
Distilled water	1 liter

The addition of 2/1000 or 3/1000 atomic weight of either aluminium or manganese to one liter of nutrient solution causes toxic action. The addition of 1/1000 atomic weight of either element results in stimulation; the stimulation is greater when 1/2000 atomic weight of both aluminium and manganese are added to the nutrient solution than when the same concentration of either element is added alone. Stoklasa's results are as follows:

Composition of solution	Dry weight of ten plants (in grams)				
	Wheat	Rye	Oats	Barley	Buckwheat
Nutrient solution	53.8	78.8	66.2	62.3	15.8
Nutrient solution+1/1,000 at. wt.*Al	56.1	80.9	68.0	67.1	20.2
Nutrient solution+1/1,000 at. wt. Mn	55.9	82.1	68.6	68.8	20.3
Nutrient solution+1/1,000 at. wt. Al+ 1/1,000 at. wt. Mn	50.0	65.2	60.9	61.3	11.8
Nutrient solution+1/2,000 at. wt. Mn	62.8	86.0	70.0	75.2	21.4
Nutrient solution+1/2,000 at. wt. Al	59.8	85.8	71.3	72.8	21.1
Nutrient solution+1/2,000 at. wt. Mn+ 1/2,000 at. wt. Al	82.2	93.6	78.5	89.4	26.5

* Atomic weight.

Sulfate of aluminium and manganese were used in the above experiments. The results were similar, however, when either chlorids, nitrates, or sulfates were employed.

McCallum (1909) reports that the treatment of seed potatoes with manganese chlorid before planting accelerates the formation of tubers. The foliage is not influenced by this treatment.

Brenchley (1910) grew barley to maturity in nutrient solutions to which varying amounts of manganese sulfate were added. The full nutrient solution was made up as follows:

KNO_3	0.50 gram
KH_2PO_4	0.25 gram
$MgSO_4$,	0.25 gram
$CaSO_4$	0.25 gram
NaCl	0.10 gram
$FeCl_3$	Trace
Distilled water	1,000 cubic centimeters

The cultures employed and the dry weights obtained are given in the following table:

Culture	Relation of $MnSO_4$ to amount of solution	Weight of shoots (grams)	Weight of roots (grams)	Weight of grain (grams)	Total weight (grams)
1	1 to 1,000	26.7	4.4	3.8	34.9
2	1 to 100,000	37.1	5.0	6.0	48.1
3	1 to 1,000,000	40.5	5.9	1.7	48.1
4	1 to 5,000,000	47.9	5.8	4.6	58.3
5	1 to 10,000,000	45.9	6.6	6.4	58.9
6	Control	39.1	6.3	7.4	52.8

At the time of harvest the seeds were ripe in culture 3. In culture 2 some seeds were ripe, others were partially ripe, and some were green. Brenchley's conclusions from these experiments are:

"1. The higher concentrations of manganese sulphate have a decided retarding effect upon the ripening of the grain.

"2. While strong solutions of manganese sulphate exert a toxic influence upon the growth of barley, very dilute solutions have a definite stimulative effect."

In another series reported by Brenchley, the nutrient solution employed was about twice as concentrated as that in the former experiment (unfortunately the exact concentration was not reported). In the stronger nutrient solution the presence of manganese in the proportion of 1 part per 10,000 of nutrient solution resulted in stimulation, the toxicity of the manganese being thus reduced.

EXPERIMENTAL STUDIES

The writer has made experiments with manganese from time to time since the autumn of 1910. The results of these experiments are presented in this bulletin. The studies may be grouped as follows:

I. The toxicity of manganese in (A) distilled water, (B) full nutrient solution, and (C) soil cultures; and (D) the relation of previous conditions of growth to toxicity.

II. The influence of light on toxicity of manganese.

III. The antidotal relations that exist between manganese and each of the minerals calcium, potassium, sodium, and magnesium.

Generally Canada field pea seedlings were employed, but in some cases, specially mentioned, wheat seedlings also were used as indicators. The manipulation and the method of experiment, unless otherwise specified were the same as those described previously.

Toxicity of manganese

Many substances at high dilution have a stimulating action toward green plants, but at higher concentrations may be poisonous. Manganese belongs to this class of substances.

Manganese salts in distilled water.— The soluble salts of manganese in distilled water are, of course, more toxic to Canada field pea seedlings than is any nutrient salt. A solution of N/4000 $MnCl_2$ is injurious, and N/2000 $MnCl_2$ prevents root growth entirely. The data presented below were obtained from pea seedlings placed in pure solutions of manganese chlorid:

TABLE 1. EXPERIMENT CONTINUED FOR FIVE DAYS. DATA FOR TEN PLANTS

Composition of solution	Average length of tops (centimeters)	Average length of roots (centimeters)
N/2,000 $MnCl_2$	0	0
N/3,000 $MnCl_2$	Slight	0
N/4,000 $MnCl_2$	1.5	2.5
N/10,000 $MnCl_2$	2.0	3.5
Distilled water	2.0	3.0

From the above table it is seen that a solution of N/3000 $MnCl_2$ prevents root growth, and that N/4000 represents the greatest dilution causing injury during the five-days exposure.

Manganese salts in full nutrient solution.— The poisonous action of manganese toward pea seedlings is greatly reduced when the solvent is a nutrient solution. A number of cultures were set up, in which nutrient solutions were made as follows:*

$Ca(NO_3)_2$	4.0	grams
KH_2PO_4	1.0	gram
KNO_3	1.0	gram
$MgSO_4$	1.0	gram
KCl	0.5	gram
$FeCl_3$	0.1	gram
Distilled water	3.0	liters

In the above nutrient solution the lethal concentration of manganese chlorid is four hundred times as strong as in distilled water.

TABLE 2. EXPERIMENT IN FULL NUTRIENT SOLUTION, CONTINUED FOR THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution	Green weight of tops (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/4 $MnCl_2$	0	0	0
N/5 $MnCl_2$	0	0	0
N/10 $MnCl_2$	0.64	0.5	5.0
N/20 $MnCl_2$	0.85	1.0	8.0
N/50 $MnCl_2$	1.50	1.0	15.0
N/100 $MnCl_2$	2.20	3.0	13.0
N/200 $MnCl_2$	3.20	6.0	13.0
N/500 $MnCl_2$	7.20	14.0	12.0
Nutrient solution	10.40	13.0	10.0

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* All full nutrient solutions employed in the experiments that follow, unless otherwise indicated, were composed as given here.

A study of the preceding table and of Fig. 9 reveals some interesting and unusual phenomena. The top growth of the seedlings amounts to only one centimeter in N/50 $MnCl_2$, nutrient solution being the solvent. In N/200 the length of stems is reduced one half, whereas the length of roots in N/50 is greater than in the control. The main roots are longer, but the laterals are shorter and less numerous, than those on the control plants. The presence of N/100 $MnCl_2$ has very little influence on the root development.

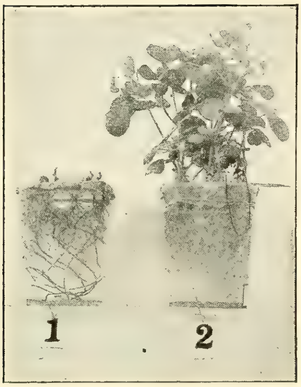

Fig. 9.— *Toxicity of* $MnCl_2$
1. N/50 $MnCl_2$ in nutrient solution
2. Control, full nutrient solution

Chlorosis of the leaves is the first indication of an overdose of manganese. The stem and leaf development of seedlings in N/300 to N/500 is normal for the first ten to fifteen days; at about the end of this time the green color begins to fade. In the stronger solutions, as in N/50 to N/200, chlorophyll does not form normally. The leaves in these cultures are chlorotic when they appear and they soon cease growing.

Manganese salts in soil cultures.— Much interest has been manifested in the stimulating action of manganese when applied to soils, as evidenced by the numerous investigations reported. On the other hand, studies of the poisonous action of manganese in soils are fewer in number; therefore the toxicity of this ion has been considered of sufficient importance for further investigation.

In order to determine the toxic action in soil, cultures were made up as indicated in Table 3. One thousand grams of air-dry sandy loam was placed in paraffined pots. To each of these pots was added a solution of $MnCl_2$ (330 cubic centimeters), as given in the table. Melted paraffin was poured over the surface of the soil, and roots of pea seedlings were inserted through holes in the paraffin into the soil below. In general the results obtained are similar to those derived from the full nutrient solutions.

The injurious action of manganese with respect to peas in soil cultures is manifested mainly toward the tops; only relatively concentrated solutions are harmful to the roots of the above plants. When a strong dose of manganese (330 cubic centimeters of N/10 $MnCl_2$ or $MnSO_4$) is applied to this particular soil, the development of the part of the plants above the surface is 1.5 centimeter, but the root growth is considerable as is shown in the table below. The injury to the roots of the plants is insignificant in the cultures that contain N/50 $MnCl_2$. Slight stimulation results from the addition of a solution of N/500 $MnCl_2$ to this soil.

TABLE 3. EXPERIMENT IN SOIL CULTURES, CONTINUED FOR THIRTY DAYS. DATA FOR TEN PLANTS

Culture	Composition oi solution	Average length of tops (centimeters)	Average length of roots (centimeters)	Weight of roots (grams)	Color of tops
1	N/5 $MnCl_2$	0	Very slight	Not taken	———
2	N/10 $MnCl_2$	1.5	6.0	3.75	Yellow
3	N/25 $MnCl_2$	10.0	8.7	5.00	Yellow
4	N/50 $MnCl_2$	12.5	12.5	8.20	Pale green
5	N/500 $MnCl_2$	16.0	13.0	10.40	Green
6	Control	15.0	12.5	9.10	Green

Chlorosis of the leaves of the plants is noticeable in all the soil cultures except cultures 5 and 6. Chlorophyll apparently does not develop in the leaves of the plants grown in soils dosed as heavily as are cultures 2 and 3. In pot 4 the yellow, or loss of green color of the leaves, is not observable until the plants are ten to fifteen days old. The loss of the green color is doubtless the result of an accumulation of manganese in the leaves; hence, in the soil cultures that contain less concentrated solutions of this element, chlorosis does not appear for several days. The chlorotic leaves do not function in carbon assimilation and death of the plants soon results.

It has been shown recently that this chlorotic action of manganese is of considerable economic importance. Kelley (1908) attributes the "yellows" of pineapples—a condition that is met with in this crop as grown in certain districts in Hawaii — to the abundance of this element. Analyses of the soils where this trouble occurs show the presence of excessive amounts of manganese, as appears on page 172.

Relation of previous conditions of growth to toxicity.— Pea seedlings that have been grown for ten days in distilled water, tap water, and full nutrient solution, respectively, are much more resistant to the poisonous influence of manganese than are those that are transferred from germinating pans and placed immediately in solutions of manganese. The nature of the medium used in this preliminary treatment — that is, whether distilled water, tap water, or full nutrient solution — has no visible effect on the resisting power of the plants as subsequently subjected to manganese treatment. In these experiments the roots were carefully rinsed with distilled water when they were removed from the tap water and the full nutrient cultures. The data, which are arranged in the following table, were taken five days after the plants were transferred to the solutions of manganese:

TABLE 4. Toxicity of Manganese after Ten Days Growth in Various Media

Ten days in distilled water, then in	Condition	Ten days in tap water, then in	Condition	Ten days in full nutrient solution, then in	Condition
N/25 $MnCl_2$	Dead	N/25 $MnCl_2$	Dead	N/25 $MnCl_2$	Dead
N/50 $MnCl_2$	Dead	N/50 $MnCl_2$	Dead	N/50 $MnCl_2$	Dead
N/75 $MnCl_2$	Dead	N/75 $MnCl_2$	Slight growth, dead	N/75 $MnCl_2$	Dead
N/100 $MnCl_2$	Slight growth, dead	N/100 $MnCl_2$	Slight growth, dead	N/100 $MnCl_2$	Slight growth, dead
N/200 $MnCl_2$	Growing	N/200 $MnCl_2$	Growing	N/200 $MnCl_2$	Growing

The lethal concentration (N/75) of manganese is practically the same for the plants that have been grown in the various media. A comparison of the data in the above table with those in Table 1 (page 179) brings out the fact that plants which have been grown for ten days in either distilled water, tap water, or full nutrient solution are approximately twenty-five times less sensitive to manganese ions than are seedlings which are placed immediately in pure solutions of manganese.

Influence of light on the injurious action of manganese with respect to plants

Certain investigators have indicated that the juice of yellow leaves — those that exhibit chlorosis resulting from the presence of excessive amounts of manganese — contains more active oxidases than does the juice of normal leaves.

Loew and Sawa (1903), who found that manganous sulfate in certain amounts is injurious to barley, state: " Manganese exerts in moderate quantities an injurious action on plants, consisting of the bleaching out of the chlorophyll. The juice of such plants shows more intense reaction for oxidases and peroxidases than the healthy control plants."

Kelley (1908) has pointed out that the leaves of pineapples grown on soils high in manganese content give more distinct reactions for oxidases with guaiacum and aloin than do the leaves from plants grown on soils containing small amounts of manganese. The writer is not able to state positively that the yellow color of the plants is brought about by actual oxidation of the chlorophyll.

It was noted in preliminary experiments that the manganese ion is especially injurious to plants grown in intense light. Later studies, from experiments conducted in cloudy weather, showed that the harmful action of manganese is retarded somewhat when the plants are grown under the lesser light intensity.

Full nutrient cultures were made in duplicate, to which varying amounts of manganese were added. One set of cultures remained in the light, the other was placed in a darkened aerated chamber; otherwise the conditions were as nearly the same as could be readily maintained. (No apparatus was at hand at the time with which the relative humidity could be regulated. This factor therefore was not controlled.) The results obtained from these experiments appear in Table 5, in Fig. 10, and in the discussion following.

In order to determine more definitely the influence of light on the toxic action of manganese, experiments were made in which plants were kept on the one hand in darkness and on the other hand in normal light. The results are given in Table 5.

It is perfectly clear that manganese is far more injurious to the tops of plants grown under ordinary greenhouse conditions than it is to those grown in darkness. In culture 1, for example, the tops of the plants were dead at the close of the experiment, while those in culture 2 were alive although the leaves were very small and the stems were greatly injured. The lower parts of the stems of the plant in the latter culture were somewhat darkened, as shown in Fig. 10. The leaves of the seed-

TABLE 5. EXPERIMENT WITH PEAS, CONTINUED FOR FIFTEEN DAYS. DATA FOR TEN PLANTS

Culture*	Composition of solution (nutrient solution as the solvent)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Remarks
1	N/25 $MnCl_2$	2.0	2.6	2	Leaves chlorotic from first. Dead at close of experiment
2	N/25 $MnCl_2$	2.0	4.3	10	Leaves little injured, darkened in places
3	N/50 $MnCl_2$	2.2	3.7	5	Leaves yellow from first. Few alive at close of experiment
4	N/50 $MnCl_2$	2.5	6.6	18	Leaves uninjured, stems slightly darkened in places
5	N/100 $MnCl_2$	2.4	4.9	7	Leaves chlorotic after three days. About one half of the leaves dead
6	N/100 $MnCl_2$	2.6	8.2	22	Leaves and stems uninjured
7	Nutrient solution	2.6	9.7	15	Leaves dark green
8	Nutrient solution	2.5	9.7	27	Leaves uninjured

* The odd numbers represent plants grown in light, the even numbers those grown in darkness.

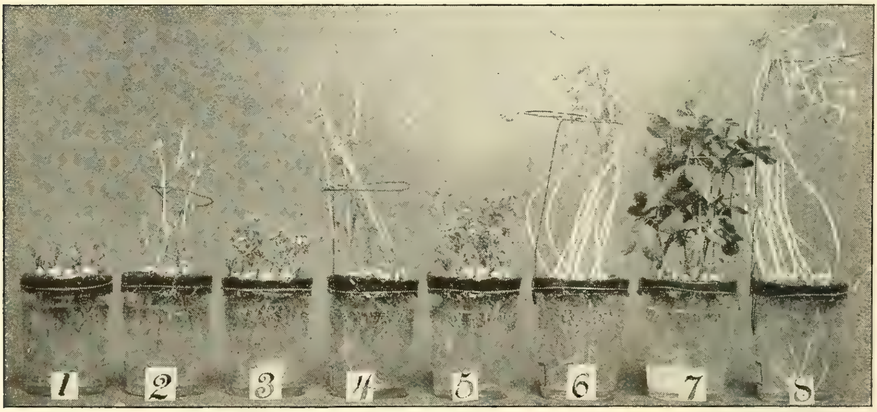

FIG. 10.— *Influence of light on toxicity of manganese*

1. N/25 $MnCl_2$ + nutrient solution in light
2. N/25 $MnCl_2$ + nutrient solution in darkness
3. N/50 $MnCl_2$ + nutrient solution in light
4. N/50 $MnCl_2$ + nutrient solution in darkness
5. N/100 $MnCl_2$ + nutrient solution in light
6. N/100 $MnCl_2$ + nutrient solution in darkness
7. Nutrient solution in light
8. Nutrient solution in darkness

lings in culture 3 became chlorotic after a short time and few remained alive until the close of the experiment; whereas those in culture 4 were uninjured and the stems were far less corroded than those in culture 2. A comparison of the plants grown in culture 6 with those in the control, culture 8, discloses the fact that the toxicity of N/100 $MnCl_2$ toward seedlings grown in the dark is very slight. On the other hand, the toxicity of manganese in ordinary greenhouse light is appreciated when one compares the development of the plants in culture 5 with that in culture 7. The writer is not prepared to offer an explanation of the cause of these results.

An examination of the data in the preceding table and of Fig. 10 further emphasizes the fact that the toxicity of manganese is manifested mainly toward the tops of the plants. The injury to the roots is inappreciable in all the above cultures except 1, 2, and 3. The increased toxicity of manganese in plants grown in light may be due to the greater accumulation of manganese in the leaves, this being brought about by greater transpiration.

Antidotal relations of calcium, potassium, sodium, and magnesium toward manganese

In a previous paper the writer has summarized some of the more important data regarding the antitoxic relations of various mineral elements as cations. He has also added the results obtained from many of his experiments. In connection with the studies on manganese, the writer has investigated the antidotal relations that exist between this element and each of the following: calcium, potassium, sodium, and magnesium.

Calcium and manganese

Calcium and manganese (*distilled water*).— By means of several series of experiments it has been definitely proved that manganese is rendered innocuous by the addition of calcium. It is to be noted, for example, that a pure solution of N/4000 $MnCl_2$ is toxic to seedlings, but when a stronger solution, N/1000 $MnCl_2$, is mixed with N/2000 $CaCl_2$ the injury due to the manganese is greatly reduced. Protective action exists also with various other combinations of calcium and manganese. The data obtained are set forth in Table 6:

TABLE 6. Experiment with Canada Field Pea, Continued for Thirty Days. Data for Ten Plants

Composition of solution	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/10 $CaCl_2$	5.90	6.15	12.05	11.0	13.0
N/25 $CaCl_2$	7.10	5.05	12.15	12.0	15.0
N/100 $CaCl_2$	6.95	4.45	11.40	12.0	12.0
N/500 $CaCl_2$	6.20	4.00	10.20	9.0	13.0
N/1,000 $CaCl_2$	6.65	3.20	9.85	12.0	12.0
N/2,000 $CaCl_2$	5.25	2.47	7.72	10.0	12.0
N/4,000 $CaCl_2$	4.25	2.10	6.35	9.0	10.0
N/10 $CaCl_2$+N/100 $MnCl_2$	2.94	5.06	8.00	7.0	13.0
N/10 $CaCl_2$+N/500 $MnCl_2$	5.46	5.81	11.27	10.0	17.0
N/10 $CaCl_2$+N/1,000 $MnCl_2$	5.12	6.92	12.04	9.0	16.0
N/10 $CaCl_2$+N/2,000 $MnCl_2$	5.15	6.20	11.35	9.0	13.0
N/10 $CaCl_2$+N/4,000 $MnCl_2$	5.76	4.10	9.86	12.0	13.0
N/25 $CaCl_2$+N/1,000 $MnCl_2$	4.52	4.20	8.72	10.0	16.0
N/100 $CaCl_2$+N/1,000 $MnCl_2$	5.56	4.10	9.66	11.0	14.0
N/500 $CaCl_2$+N/1,000 $MnCl_2$	3.75	3.50	7.25	8.0	13.0
N/2,000 $CaCl_2$+N/1,000 $MnCl_2$	3.45	3.05	6.50	6.5	13.0
N/4,000 $CaCl_2$+N/1,000 $MnCl_2$	2.01	2.40	4.41	5.0	10.0
N/1,000 $MnCl_2$*	—	—	—	—	—
N/4,000 $MnCl_2$	1.80	1.64	3.44	5.0	6.5
Distilled water	1.85	1.75	3.60	6.0	7.5

*Weight not taken. Practically no growth. Dead.

As is brought out in the above table, calcium is effective in overcoming the harmful properties of manganese. It is to be noted that solutions of N/10 $CaCl_2$ reduce the deleterious action of various amounts of this mineral element. The greatest development of plants (in mixed solutions) occurs in cultures composed of N/10 $CaCl_2$+N/1000 $MnCl_2$. In N/10 $CaCl_2$+N/100 $MnCl_2$ the toxicity of the manganese toward the tops is not reduced to such an extent as it is toward the roots. The plants in this culture were somewhat chlorotic. It is to be noted further that a very slight amount of calcium (N/4000 $CaCl_2$) reduces the injury caused by N/1000 $MnCl_2$. In no case has the addition of manganese to a solution of calcium resulted in an increased development of plants over that in the corresponding solutions of calcium alone.

The relation of previous conditions of growth to antidotal relations between calcium and manganese.— In order to determine whether the age of seedlings modifies the protective action of calcium toward manganese, seedlings were permitted to grow for ten days in pure distilled water and were then transferred to single and mixed solutions of calcium and manganese. Final notes were taken twenty days later. It was thought that changes which would alter the relations of the calcium and manganese might occur in the protoplasm of the cells of the older seedlings. The results obtained show clearly that, although the lethal concentrations of manganese are much higher for older seedlings, calcium counteracts the toxicity of this element in the same manner as before. Slight elongation of the tops takes place when the plants are placed in N/200 $MnCl_2$. A solution of N/100 causes death within three days. The addition of N/10 $CaCl_2$ to the above solutions of manganese results in complete reduction of the toxicity.

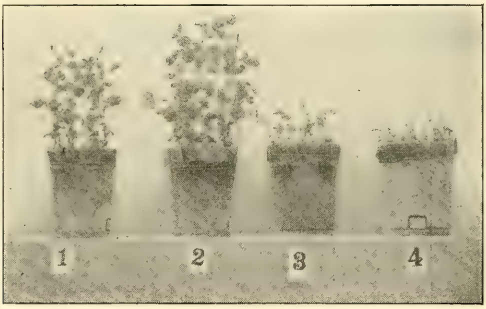

FIG. 11.— *Antidotal relations between calcium and manganese. Seedlings in distilled water for ten days, then transferred to*

1. N/10 $CaCl_2$+N/100 $MnCl_2$
2. N/10 $CaCl_2$
3. Distilled water
4. N/100 $MnCl_2$

TABLE 7. EXPERIMENT CONTINUED FOR TWENTY DAYS. DATA FOR TEN PLANTS

Previous condition of growth	Composition of solution	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
Ten days in distilled water	N/10 $CaCl_2$	9.45	15	9
	N/10 $CaCl_2$+N/100 $MnCl_2$	8.65	13	7
	N/10 $CaCl_2$+N/200 $MnCl_2$	8.82	14	8
	N/100 $MnCl_2$	Dead after three days	——	——
	N/200 $MnCl_2$	Weight not taken	Slight increase	No increase
	Distilled water	2.50	6	6

Full nutrient solution also was employed as a medium in which to grow seedlings for the first ten days. The plants were then placed in cultures containing calcium and manganese separately, and in cultures in which these metals were both in solution. (Precautions were taken to wash carefully the roots of the seedlings when they were removed from the nutrient solution.) Final notes were made twenty days later. The total and the average length of both tops and roots of these plants are greater than in those that were grown for the first ten days in distilled water and then transferred to solutions of calcium and manganese. Here again calcium ions are effective in antidoting manganese ions.

TABLE 8. Experiment Continued for Twenty Days. Data for Ten Plants

Previous condition of growth	Composition of solution	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
Ten days in full nutrient solution	N/10 $CaCl_2$	18.85	24	11
	N/10 $CaCl_2$+N/50 $MnCl_2$	10.35	20	9
	N/10 $CaCl_2$+N/100 $MnCl_2$	11.42	22	11
	N/10 $CaCl_2$+N/200 $MnCl_2$	16.55	25	10
	N/100 $MnCl_2$	No further growth	Dead	——
	N/200 $MnCl_2$	Very slight growth	——	——
	Distilled water	11.95	14	8

It may be concluded, from the data obtained from the two series of experiments, that the nature of the substratum (that is, whether distilled water or nutrient solution) in which the seedlings were grown for the first ten days does not influence markedly the antidotal relation of calcium to manganese.

Calcium and manganese (full nutrient solution).— Data have been presented which establish the fact that manganese is much less toxic with respect to seedlings in full nutrient solutions than to seedlings in distilled water. Nevertheless, the manganese ion is poisonous in cultures that contain all the essential nutrients. A number of cultures were made up in order to determine whether further antidotal relations exist between calcium and manganese when a full nutrient solution is employed as the solvent.

In general, the experiments demonstrate that calcium decreases the deleterious action of manganese in the nutrient solution indicated, as might be anticipated from the concentration of the bases. N/50 $MnCl_2$ prevents growth of tops almost entirely. In this culture the lateral roots are shorter and less numerous, but the main roots are longer, than those on the control plants.

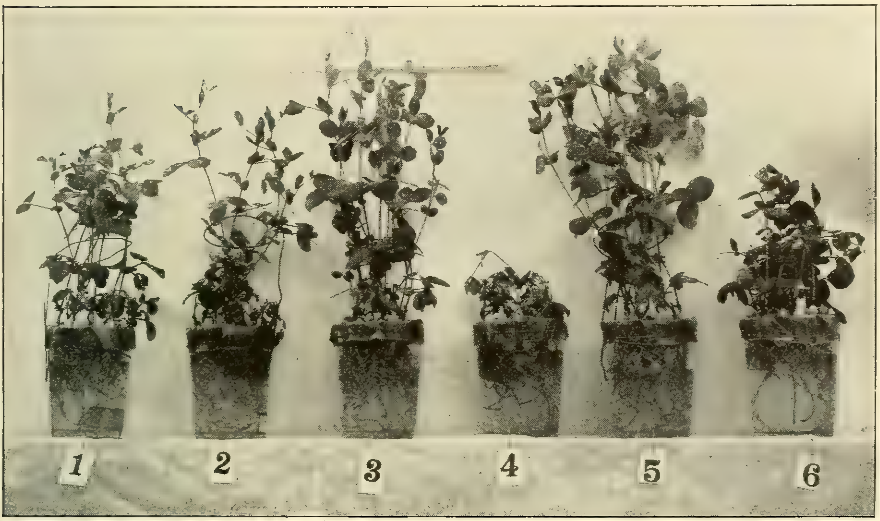

FIG. 12.— *Antidotal relations between calcium and manganese. Plants grown in full nutrient solution for ten days, then transferred to*

1. N/10 $CaCl_2$ + N/50 $MnCl_2$
2. N/10 $CaCl_2$ + N/100 $MnCl_2$
3. N/10 $CaCl_2$ + N/200 $MnCl_2$
4. N/100 $MnCl_2$
5. N/10 $CaCl_2$
6. Distilled water

The addition of sufficient $CaCl_2$ to make the solution N/10, N/50, N/100, or N/500 of the solvent, results in no reduction of the toxicity of the above solution of manganese.

N/100 $MnCl_2$ is likewise very toxic to tops, and to a less extent to roots, of seedlings. Chlorosis of the leaves soon appears in this culture. The presence of either N/10 or N/50 $CaCl_2$ appreciably counteracts this deleterious action, the loss of green color being then scarcely apparent.

The development of the roots in solutions of N/200 $MnCl_2$ is almost as extensive as in the control cultures. The length of the stems is about one half that of those grown in the nutrient solution. The presence of N/500 $CaCl_2$ results in a slight increase in elongation of the stems. The detailed data obtained appear in Table 9:

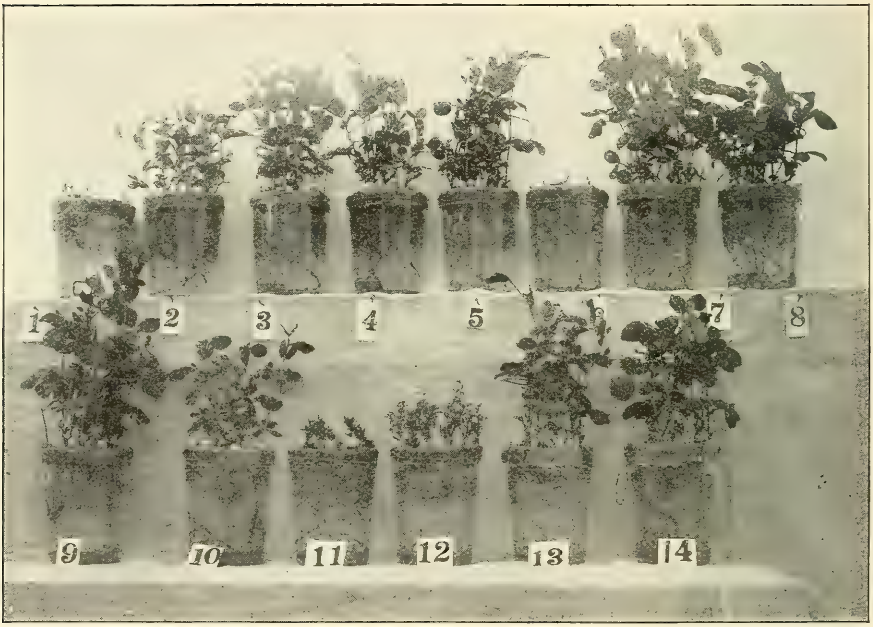

Fig. 13.— *Antidotal relations between calcium and manganese; nutrient solution as the solvent*

1. N/10 $CaCl_2$+N/50 $MnCl_2$
2. N/10 $CaCl_2$+N/100 $MnCl_2$
3. N/10 $CaCl_2$+N/500 $MnCl_2$
4. N/50 $CaCl_2$+N/500 $MnCl_2$
5. N/100 $CaCl_2$+N/500 $MnCl_2$
6. N/500 $CaCl_2$+N/50 $MnCl_2$
7. N/10 $CaCl_2$
8. N/50 $CaCl_2$
9. N/100 $CaCl_2$
10. N/500 $CaCl_2$
11. N/50 $MnCl_2$
12. N/100 $MnCl_2$
13. N/500 $MnCl_2$
14. Nutrient solution

TABLE 9. EXPERIMENT CONTINUED FOR THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution (full nutrient solution as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/10 $CaCl_2$+N/50 $MnCl_2$	0.50	1.46	1.96	1	6
N/10 $CaCl_2$+N/100 $MnCl_2$	5.05	4.95	10.00	11	8
N/10 $CaCl_2$+N/500 $MnCl_2$	7.36	6.35	13.71	13	11
N/50 $CaCl_2$+N/50 $MnCl_2$	0.45	—	—	1	15
N/50 $CaCl_2$+N/100 $MnCl_2$	2.55	5.55	8.10	4	12
N/100 $CaCl_2$+N/50 $MnCl_2$	0.35	1.75	2.10	1	11
N/100 $CaCl_2$+N/100 $MnCl_2$	2.55	4.20	6.75	5	10
N/100 $CaCl_2$+N/500 $MnCl_2$	7.19	4.85	12.04	13	11
N/500 $CaCl_2$+N/50 $MnCl_2$	0.37	2.30	2.67	1	12
N/500 $CaCl_2$+N/100 $MnCl_2$	2.10	4.90	7.00	5	14
N/500 $CaCl_2$+N/200 $MnCl_2$	3.15	4.60	7.75	7	12
N/1,000 $CaCl_2$+N/100 $MnCl_2$	1.60	4.26	5.86	2	13
N/1,000 $CaCl_2$+N/200 $MnCl_2$	2.10	3.80	5.90	4	13
N/50 $MnCl_2$	1.00	2.95	3.95	2	15
N/100 $MnCl_2$	2.25	4.00	6.25	4	13
N/200 $MnCl_2$	2.70	4.45	7.15	6	13
N/500 $MnCl_2$	6.90	4.65	11.55	14	12
N/10 $CaCl_2$	9.75	7.15	16.90	16	10
N/50 $CaCl_2$	9.10	5.60	14.70	12	12
N/100 $CaCl_2$	10.85	6.15	17.00	15	10
N/500 $CaCl_2$	10.20	5.45	15.65	14	16
N/1,000 $CaCl_2$	9.90	4.90	14.80	14	9
Full nutrient solution	10.40	5.20	15.60	13	10

Calcium and manganese (*soil cultures*).— It is of special interest and of great economic importance to ascertain whether the toxicity of manganese in soils may be reduced by the application of lime. The writer has found from repeated experiments that large applications of lime to pots of soil overdosed with manganese result in a more vigorous development of the plants in the pots.

In these experiments glass tumblers were used as containers. In each tumbler was placed 250 grams of air-dry sandy loam, to which was added 80 cubic centimeters of the solutions employed. Ten pea seedlings were used in each tumbler, the roots growing into the moist soil through a hole in the paraffin that had previously been applied to the surface of the soil. Subsequently the number of plants was reduced to five in each tumbler. The water lost from the soil was replaced from time to time by the addition of distilled water. After thirty days the experiments were discontinued. The results obtained appear in Table 10:

TABLE 10. Experiment Continued for Thirty Days

(The data observed for five plants were calculated for ten plants, so that a comparison might be made with other experiments)

Composition of solution		Total weight of tops (grams)	Total weight of roots (grams)	Average length of tops (centimeters)	Remarks
N/25 $MnCl_2$	(a)	Too dry	3.2	6	Tops dead
	(b)	to weigh	2.3	9	
N/50 $MnCl_2$	(a)	7.0	12.2	24	Leaves slightly chlorotic
	(b)	8.5	10.0	22	
Distilled water	(a)	9.0	9.0	27	Color good
	(b)	10.0	——	29	
N/25 $CaCl_2$+N/25 $MnCl_2$	(a)	5.0	5.4	18	Color fair
	(b)	5.6	4.1	20	
N/50 $CaCl_2$+N/50 $MnCl_2$	(a)	9.2	8.2	28	Color fair
	(b)	9.8	8.6	25	
N/25 $CaCl_2$	(a)	8.0	10.2	26	Color good
	(b)	6.4	7.8	25	
N/50 $CaCl_2$	(a)	8.1	10.0	28	Color good
	(b)	——	——	——	

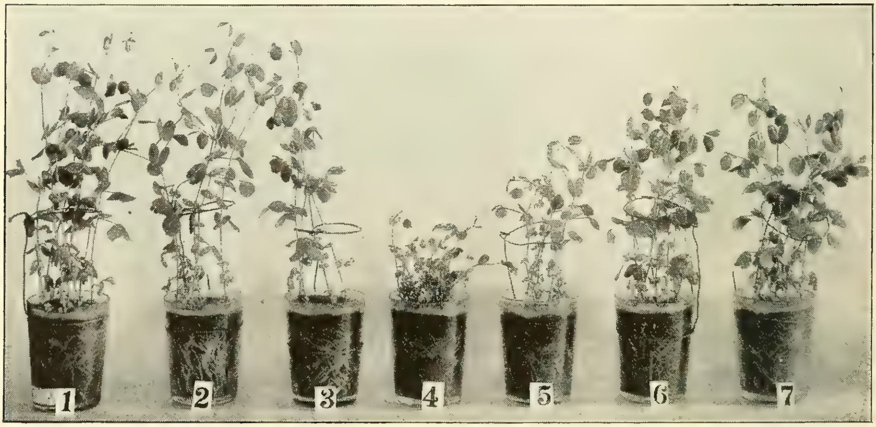

Fig. 14.— *Antidotal relations between calcium and manganese in soil cultures*

1. N/50 $CaCl_2$
2. N/50 $CaCl_2$+N/50 $MnCl_2$
3. N/50 $MnCl_2$
4. N/25 $MnCl_2$
5. N/25 $MnCl_2$+N/25 $CaCl_2$
6. N/25 $CaCl_2$
7. Distilled water

The addition of N/25 $MnCl_2$ to this particular soil results in great injury to pea seedlings. The growth of tops is only 6 centimeters and the root growth is likewise depressed. The presence of N/25 $CaCl_2$ reduces the injury from the manganese, as the average growth of tops in this culture is 19 centimeters and the root growth is also more extensive. These results are at variance with those obtained by pineapple-growers in Hawaii, as Kelley (1908) reports that the addition of lime or other fertilizers is not effective in counteracting the injury resulting from manganese.

Potassium and manganese

Potassium and manganese (distilled water).— Since calcium is relatively effective in antidoting manganese, it is of interest to ascertain whether other metals as cations counteract this injurious action. By means of several sets of experiments it has been determined that mutual antagonism exists between manganese and each of the following: potassium, sodium, and magnesium. N/500 $MnCl_2$ prevents growth of wheat seedlings and N/2000 is extremely toxic. This deleterious action is overcome by the presence of N/50 KCl. A weaker solution of potassium, N/500, is antagonistic toward manganese, but to a less extent than more concentrated solutions.

N/50 KCl is very injurious to roots of wheat seedlings. The presence of either N/500, N/2000, or N/5000 $MnCl_2$ counteracts this toxicity. An examination of Table 11 bears out the above statements:

TABLE 11. EXPERIMENT WITH WHEAT, CONTINUED FOR THIRTY DAYS. DATA FOR EIGHT PLANTS

Composition of solution	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 KCl	0.65	6	1
N/50 KCl+N/500 $MnCl_2$	0.85	8	3
N/50 KCl+N/2,000 $MnCl_2$	1.50	9	4
N/50 KCl+N/5,000 $MnCl_2$	1.20	11	3
N/500 KCl+N/2,000 $MnCl_2$	0.80	9	2
N/2,000 $MnCl_2$	0	2	0
Distilled water	0.55	6	4

Potassium and manganese (N/1000 $CaCl_2$).— Under ordinary greenhouse conditions pea seedlings, in distilled water or in full nutrient solution lacking calcium, rarely grow to a height of more than two inches. However, if calcium is present, even in relatively small amounts, growth proceeds for about four weeks. The ash of pea seeds contains relatively small amounts of calcium, but the magnesia content is high. This unbalanced condition probably accounts for the demands for calcium in the above instances. Taking cognizance of these facts, N/1000 $CaCl_2$ was used as the solvent in making up cultures in order to determine whether, under such circumstances, antidotal relation may be recognized to exist between potassium and manganese. The solutions used and the results obtained appear in the following table:

TABLE 12. Experiment Continued for Thirty Days. Data for Ten Plants

Composition of solution (N/1000 $CaCl_2$ as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 KCl	3.75	5.05	6	9
N/50 KCl+N/100 $MnCl_2$	0.45	2.01	1	6
N/50 KCl+N/500 $MnCl_2$	4.10	4.08	8	8
N/50 KCl+N/1,000 $MnCl_2$	5.10	4.25	9	10
N/500 KCl+N/1,000 $MnCl_2$	3.35	4.02	7	10
N/1,000 KCl+N/1,000 $MnCl_2$	3.16	3.15	6	12
N/1,000 $MnCl_2$	2.45	3.35	5	10
N/1,000 $CaCl_2$	5.01	3.35	8	10

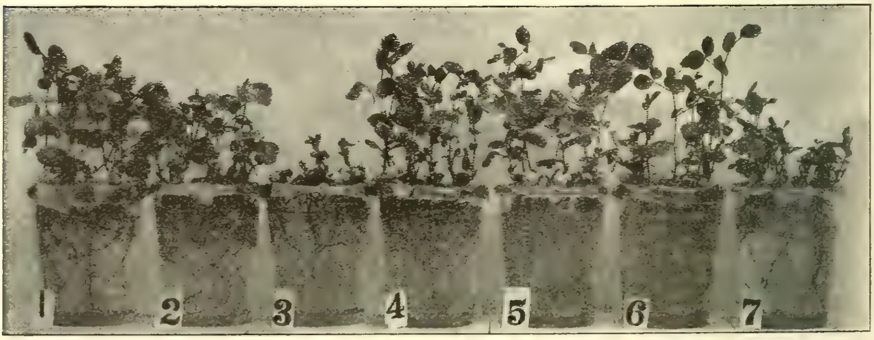

Fig. 15.—*Antidotal relations between potassium and manganese; N/1000 $CaCl_2$ as the solvent*

1. N/1000 $CaCl_2$
2. N/50 KCl
3. N/50 KCl+N/100 $MnCl_2$
4. N/50 KCl+N/500 $MnCl_2$
5. N/50 KCl+N/1000 $MnCl_2$
6. N/500 KCl+N/1000 $MnCl_2$
7. N/1000 $MnCl_2$

The root development of the plants in the culture composed of N/1000 $CaCl_2$+N/50 KCl is far more abundant than in the control, N/1000 $CaCl_2$, but the top growth is less. It is thus seen that N/50 KCl depresses the growth of the stems. When either N/500 or N/1000 $MnCl_2$ is present with N/50 KCl, in the presence of the weak calcium, the retarding action of the potassium is entirely overcome.

Manganese is antidoted by potassium, as is shown by Fig. 15 and by the data in Table 12. Slight antagonism results in cultures composed of N/50 KCl+N/100 $MnCl_2$+N/1000 $CaCl_2$. Greater protective action exists when the above amount of potassium is present with either N/500 or N/1000 $MnCl_2$. Considering the top growth of seedlings, mutual antagonism exists between potassium and manganese; considering root growth alone, however, potassium has a protective action toward manganese.

Potassium and manganese (full nutrient solution).— The further addition of potassium to a full nutrient solution one tenth as strong as the one employed in the previous experiments, lowers the deleterious action of either N/100 or N/500 $MnCl_2$. The further addition of potassium (N/20 KCl) to the nutrient solution also results in an increase of root development, the lateral roots especially being longer and more numerous than those of the control plants. On the other hand, the top growth is retarded somewhat. The results obtained from these experiments appear in Table 13:

TABLE 13. EXPERIMENT CONTINUED FOR THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution (full nutrient solution as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/20 KCl	4.85	4.26	9.11	10.0	5.6
N/20 KCl+N/100 $MnCl_2$	1.45	1.68	3.13	6.0	7.5
N/20 KCl+N/500 $MnCl_2$	4.60	3.50	8.10	9.0	6.0
N/100 $MnCl_2$	0.85	1.35	2.20	1.5	5.0
N/500 $MnCl_2$	2.15	3.20	5.35	6.0	7.0
Full nutrient solution	5.15	3.55	8.70	13.0	4.0

Sodium and manganese

Sodium and manganese (*distilled water*).— Sodium and manganese are mutually antagonistic in distilled water. Solutions of N/50 NaCl reduce the poisonous action of solutions of manganese ranging in concentration from N/500 to N/5000. The toxicity of both sodium and manganese toward wheat seedlings is not evident when the salts of these metals are present in solution as N/50 NaCl+N/5000 $MnCl_2$, and the amount of injury is greatly lessened in the other combinations employed. The data obtained are summarized in Table 14:

TABLE 14. Experiment with Wheat, Continued for Thirty Days. Data for Eight Plants

Composition of solution	Total weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
N/50 NaCl+N/500 $MnCl_2$	0.36	2.0	1.0
N/50 NaCl+N/1,000 $MnCl_2$	0.80	5.5	2.0
N/50 NaCl+N/3,000 $MnCl_2$	0.90	6.0	8.0
N/50 NaCl+N/5,000 $MnCl_2$	0.92	9.0	9.0
N/50 NaCl	Very slight	1.0	0
N/3,000 $MnCl_2$	0.48	3.0	2.0
N/5,000 $MnCl_2$	0.55	4.0	3.0
Distilled water	0.92	9.0	4.0

Magnesium and manganese

Magnesium and manganese (*N/1000* $CaCl_2$).— The fact that magnesium and manganese are very toxic in pure solutions makes it especially interesting to ascertain the results when these are combined. The development of pea seedlings is greater in cultures containing both these harmful substances than in cultures in which only one is present, again indicating mutual antagonism. N/500 and N/1000 $MnCl_2$, for example, are injurious to the tops. This injury is prevented by the presence of N/50, N/100, or N/200 $MgCl_2$; moreover, the development of the plants in these solutions is more extensive than in the corresponding solutions of magnesium.

Magnesium and manganese (*full nutrient solution*).— In this series, as in the preceding one, magnesium effectively counteracts the injurious action of manganese toward pea seedlings. It is to be noted, for example, that solutions of N/200 $MnCl_2$ are very toxic; the leaves of seedlings

TABLE 15. EXPERIMENT CONTINUED FOR TWENTY-FIVE DAYS. DATA FOR EIGHT PLANTS

Composition of solution ($N/1000$ $CaCl_2$ as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Total green weight (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)
$N/50$ $MgCl_2$	2.10	1.20	3.30	6.0	7.0
$N/100$ $MgCl_2$	2.75	1.84	4.59	8.0	9.5
$N/200$ $MgCl_2$	3.62	2.04	5.66	13.0	12.0
$N/50$ $MgCl_2+N/500$ $MnCl_2$	4.80	2.40	7.20	13.5	12.5
$N/100$ $MgCl_2+N/500$ $MnCl_2$	4.75	2.50	7.25	12.0	13.0
$N/200$ $MgCl_2+N/500$ $MnCl_2$	4.00	2.30	6.30	11.0	13.0
$N/100$ $MgCl_2+N/1{,}000$ $MnCl_2$	4.20	2.00	6.20	16.0	14.0
$N/200$ $MgCl_2+N/100$ $MnCl_2$	4.90	2.50	7.40	14.0	14.0
$N/500$ $MnCl_2$	1.75	1.65	3.40	7.0	12.0
$N/1{,}000$ $MnCl_2$	2.54	1.75	4.29	10.0	13.0
$N/1{,}000$ $CaCl_2$	3.57	1.70	5.27	12.0	10.0

grown in these cultures become chlorotic and the top growth is reduced. The addition of $N/20$ $MgCl_2$ prevents this injury. Moreover, the presence of $N/50$ $MgCl_2$ prevents chlorosis which appears in plants grown in solutions of $N/500$ $MnCl_2$.

TABLE 16. EXPERIMENT CONTINUED FOR TWENTY-FIVE DAYS. DATA FOR EIGHT PLANTS

Composition of solution (full nutrient solution as the solvent)	Green weight of tops (grams)	Green weight of roots (grams)	Average length of tops (centimeters)	Average length of roots (centimeters)	Remarks
$N/200$ $MnCl_2$	3.10	2.60	8.0	10.0	Lateral roots long, leaves yellow
$N/500$ $MnCl_2$	6.20	3.00	15.0	12.0	Lateral roots long, leaves yellowish
$N/50$ $MgCl_2$	7.15	3.50	13.0	14.0	Lateral roots short, leaves dark green
$N/20$ $MgCl_2+N/200$ $MnCl_2$	6.05	2.80	17.5	18.0	Lateral roots short, color of leaves good
$N/50$ $MgCl_2+N/200$ $MnCl_2$	4.10	2.75	16.0	16.0	Lateral roots long, color of leaves pale
$N/50$ $MgCl_2+N/500$ $MnCl_2$	7.27	3.40	17.0	13.0	Lateral roots medium, color of leaves good
Nutrient solution	7.27	3.60	14.0	13.0	Lateral roots medium, color of leaves good

CONCLUSIONS

Pure solutions of manganese salts are extremely poisonous to pea and wheat seedlings. The degree of toxicity is greatly reduced by full nutrient solutions and by soil cultures.

The injurious action of the manganese ion is manifested mainly toward the tops of plants. Chlorosis of the leaves is the first indication of an overdose of manganese.

Manganese is less injurious to plants grown in the dark than to those grown in the light.

Calcium, potassium, sodium, and magnesium ions are each effective in counteracting the poisonous action of manganese. Mutual antagonism exists between the manganese ion and each of the following: potassium, sodium, and magnesium.

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THE ACTION OF CERTAIN NUTRIENT AND NON-NUTRIENT BASES ON PLANT GROWTH — III

TOXICITY OF VARIOUS CATIONS*

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HISTORICAL

As a result of numerous investigations that have been made on antagonistic action and toxic action, there are available for green plants many data with respect to the relative toxicity of certain nutrient and non-nutrient substances. In the first part of this paper extensive data have been reviewed and presented regarding the modification of toxic action in mixed solutions, especially the effects of the cations, one on another, of the substances constituting the nutrient solution. Furthermore, the experiments of Guthrie and Helms (1903–1905), Kanda (1904), Cameron and Breazeale (1904), Dandeno (1904), Harter (1905), True and Oglevee (1905), Jensen (1907), Magowan (1908), Duggar (1911), and others have shown that, as a rule, substances are far less toxic in the presence of inert solid particles, sand, or soil, than when present in pure solutions.

The reduction of toxicity by quartz sand, soil, and other solid particles has been attributed to the following factors: chemical reactions between the solute and the solid particles, resulting in the formation of less toxic substances; adsorption of the molecules or ions of the toxic agent; and obstruction to the free movement of the molecules or ions in solution.

The reduction of the toxicity of deleterious agents when present in nutrient solutions may be attributed in part to chemical reactions that result in the formation of less toxic substance, to a decrease in penetrability of the protoplasmic membrane, and perhaps to other important factors.

EXPERIMENTAL

The writer has made comparative studies of the poisonous action of the chlorids of calcium, potassium, sodium, magnesium, ammonium, barium, strontium, and manganese, in distilled water, in full nutrient solution, and in soil cultures. The concentrations in which no further elongation of seedlings takes place, those in which slight growth occurs, and those that result in no injury to seedlings, have been determined. As a rule, Canada field pea seedlings were employed as indicators, although in some instances wheat seedlings also were used.

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The methods of experiment and manipulation were the same as those described in Part I.

Toxicity of calcium

Each of the other elements studied is far more toxic than calcium. In fact, calcium ions do not prohibit growth of pea and wheat seedlings at concentrations much below those that result in plasmolysis, although at the higher concentrations some injury ensues. The results that were obtained from the experiments are summarized in the tables immediately following:

TABLE 1. Experiment with Canada Field Pea. Duration, Ten Days. Data for Ten Plants

Composition of solution	Pea		Wheat	
	Increase in length of tops	Increase in length of roots	Increase in length of tops	Increase in length of roots
Distilled water as the solvent				
N/2 $CaCl_2$	None	None	None	None
N/3 $CaCl_2$	Slight	Slight	None	None
N/5 $CaCl_2$	Slight	Slight	Slight	Slight
N/10 $CaCl_2$	Good	Good	Good	Good
Distilled water	Good	Good	Good	Good
Full nutrient solution as the solvent				
N/2 $CaCl_2$	None	None	None	None
N/4 $CaCl_2$	Slight	Slight	Slight	Slight
N/5 $CaCl_2$	Slight	Slight	Slight	Slight
N/10 $CaCl_2$	Good	Good	Good	Good
Nutrient solution	Good	Good	Good	Good
Soil cultures*				
N/1 $CaCl_2$	None	None	None	None
N/2 $CaCl_2$	None	None	None	None
N/4 $CaCl_2$	2.0 cm.	2.5 cm.	5.1 cm.	2.5 cm.
N/5 $CaCl_2$	4.0 cm.	5.1 cm.	10.2 cm.	6.3 cm.
Water	5.1 cm.	5.1 cm.	———	———

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* Air-dry soil, to which was added a quantity of the solution equal to thirty per cent of its dry weight. The soil employed was a sandy loam containing 3.5 per cent volatile matter.

Toxicity of potassium

Potassium is appreciably more toxic than calcium, but less injurious than the other ions that the writer has studied. As is the case with calcium, the injury is reduced when potassium is present in full nutrient solution and in soil cultures. Slight elongation of tops occurs in solutions composed of N/10 KCl, but no appreciable root growth appears in solutions stronger than N/50 KCl. Cameron and Breazeale (1904) obtained similar results from other substances; for instance, they found that "the concentration required to kill a seedling or disorganize an entire radicle varies widely from that required to completely hinder any growth or elongation, or that which will permit some elongation . . . . . ." The results of the writer's studies appear in Table 2:

TABLE 2. Experiment with Wheat and Pea Seedlings, Continued for Ten Days. Data for Ten Plants

Composition of solution	Pea		Wheat	
	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)
Distilled water as the solvent				
N/5 KCl	0	0	0	0
N/10 KCl	0.25	0	0	0
N/20 KCl	0.50	0	6.00	0
N/30 KCl	1.00	0	6.00	0
N/50 KCl	3.00	Slight	7.00	2.00
N/200 KCl	6.00	7.50	6.50	7.00
Distilled water	6.00	7.00	7.00	6.00
Full nutrient solution as the solvent				
N/4 KCl	0	0	2.50	0
N/5 KCl	2.50	Slight	5.00	1.50 No branches
N/7 KCl	5.00	3.75	6.25	6.25 Few branches
N/10 KCl	6.25	7.50	7.50	7.50 Slightly branched
N/50 KCl	7.50	10.00	8.75	11.25
Nutrient solution	6.25	8.75	7.50	10.00 Branched

Toxicity of sodium

Sodium ions in distilled water are somewhat more deleterious to pea and wheat seedlings than are potassium ions. No top growth results in solutions stronger than N/30 NaCl, whereas slight development of tops occurs in cultures containing N/10 KCl. The lethal concentration of sodium for roots is just below N/75.

TABLE 3. Experiment with Pea and Wheat Seedlings, Continued for Ten Days. Data for Ten Plants

Composition of solution	Pea		Wheat	
	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)
Distilled water as the solvent				
N/5 NaCl	0	0	0	0
N/10 NaCl	0	0	0	0
N/20 NaCl	0	0	0	0
N/30 NaCl	0.25	0	0	0
N/50 NaCl	0.50	0	1.00	0
N/75 NaCl	3.00	3.00	2.00	3.50
Distilled water	6.00	7.00	7.00	8.00
Full nutrient solution as the solvent				
N/3 NaCl	0	0	——	——
N/4 NaCl	0	0	0	0
N/5 NaCl	1.00	2.00	1.00	2.00
N/10 NaCl	6.00	7.00	9.00	8.00
Nutrient solution	7.50	8.50	9.00	10.00
Soil cultures				
N/1 NaCl	0	0	——	——
N/2 NaCl	0	0	——	——
N/3 NaCl	——	——	——	——
N/5 NaCl	Slight	Slight	——	——
N/10 NaCl	6.50	7.00	——	——
Distilled water	6.00	6.50	——	——

Harter (1905) gives .054 N as the average limit of pure solutions of sodium chlorid for several varieties of wheat. In his experiments the roots of the seedlings were in contact with the solutions only twenty-four hours. This undoubtedly accounts for the higher values that he obtained.

Toxicity of magnesium

Magnesium has long since been considered as injurious to plants when calcium is absent from the media or present in relatively small amounts. It is to be noted that the lethal concentration of magnesium ions in distilled water with respect to the roots of seedlings is at a dilution several times greater than that of either sodium or potassium. Wheat seedlings are more resistant to this element than are pea seedlings. No root growth occurs with peas in solutions composed of N/500 $MgCl_2$, but slight elongation of the roots of wheat seedlings takes place. The following data verify the above statements:

TABLE 4. EXPERIMENTS WITH PEA AND WHEAT SEEDLINGS, CONTINUED FOR THIRTY DAYS. DATA FOR TEN PLANTS

Composition of solution	Pea		Wheat	
	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)
Distilled water as the solvent				
N/50 $MgCl_2$	2.0	0	0.5	0
N/100 $MgCl_2$	2.0	0	4.0	0
N/300 $MgCl_2$	3.0	0	6.0	0
N/500 $MgCl_2$	3.5	0	7.0	2.0
N/1,000 $MgCl_2$	5.0	Slight-dead	7.0	6.0
Distilled water	6.0	7.0	7.0	6.0
Full nutrient solution as the solvent				
N/4 $MgCl_2$	0	0	0	0
N/5 $MgCl_2$	2.0	Slight	Slight	1.0
N/6 $MgCl_2$	3.0	1.5	3.0	4.0
N/10 $MgCl_2$	6.0	7.0	7.0	8.0
Nutrient solution	7.5	8.5	9.0	10.0

In working with several varieties of wheat, Harter (1905) ascertained that considerable differences exist between the varieties in their power to resist the poisonous action of various ions. In determining the limit for any one variety, he took as the lethal concentration the one in which about one half of the seedlings lived and one half died after remaining in the solutions for twenty-four hours. The average lethal concentration for magnesium chlorid was found to be N/110 (.0093 N), which is much more concentrated than the values obtained by the writer for seedlings that remained in the solutions for ten days.

Toxicity of ammonium

Roots of both pea and wheat seedlings are especially sensitive to ammonium in distilled water, slight elongation of the roots of either taking place in N/1000 NH_4Cl. However, much stronger solutions are required in order to prevent top growth; for example, top growth is evident in solutions of N/20 NH_4Cl.

TABLE 5. Experiments with Pea and Wheat Seedlings. Duration, Ten Days. Data for Ten Plants

Composition of solution	Pea		Wheat	
	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)
Distilled water as the solvent				
N/5 NH_4Cl	0	0	0	0
N/10 NH_4Cl	0	0	0	0
N/20 NH_4Cl	0.5	0	1.0	0
N/30 NH_4Cl	1.0	0	1.5	0
N/50 NH_4Cl	1.0	0	3.0	0
N/100 NH_4Cl	2.0	Very slight	5.0	Very slight
N/500 NH_4Cl	3.0	Very slight	8.0	2.0
N/1,000 NH_4Cl	3.0	Very slight	8.0	2.5
Distilled water	6.0	7.5	7.0	6.5
Full nutrient solution as the solvent				
N/3 NH_4Cl	0	0	—	—
N/4 NH_4Cl	Slight	1.5	—	—
N/5 NH_4Cl	Slight	3.0	—	—
N/10 NH_4Cl	2.5	4.0	—	—
Nutrient solution	7.5	8.5	—	—
Soil cultures				
N/3 NH_4Cl	0	0	—	—
N/5 NH_4Cl	3.0	2.5	—	—
N/10 NH_4Cl	5.5	4.0	—	—
Distilled water	7.0	7.5	—	—

Toxicity of barium

The writer has presented data by means of which it was demonstrated that barium ions when present in distilled water are extremely deleterious to pea seedlings. The data given below lend further emphasis to this point. Although N/4000 $BaCl_2$ is the lethal concentration for roots of pea seedlings, great injury results from far more dilute solutions. It will be noted in Table 6 that N/10,000 $BaCl_2$ retards root development. In a full nutrient solution the same root retardation is secured only with N/20 $BaCl_2$. The toxicity of barium is of course greatly depressed when present in full nutrient solutions or in soil cultures.

TABLE 6. EXPERIMENT WITH CANADA FIELD PEA SEEDLINGS. DURATION, TEN DAYS. DATA FOR TEN PLANTS

Composition of solution	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)
Distilled water as the solvent		
N/3,000 $BaCl_2$	2.5	0
N/4,000 $BaCl_2$	4.0	0
N/5,000 $BaCl_2$	4.5	Slight
N/10,000 $BaCl_2$	6.0	2.5
Distilled water	6.0	7.5
Full nutrient solution as the solvent		
N/6 $BaCl_2$	0	0
N/10 $BaCl_2$	0	0
N/20 $BaCl_2$	2.0	2.5
N/100 $BaCl_2$	6.0	5.0
Control	7.5	8.5

Toxicity of strontium

Strontium exhibits a rather unusual behavior toward seedlings. Comparatively dilute solutions in distilled water are extremely injurious, but very strong solutions are required in order to prevent growth. For example, N/2000 $SrCl_2$ greatly retards the growth of the roots of pea seedlings, but roots are not killed when placed in solutions of N/7 $SrCl_2$, slight development resulting.

TABLE 7. EXPERIMENT WITH CANADA FIELD PEA SEEDLINGS. DURATION, TEN DAYS. DATA FOR TEN PLANTS

Composition of solution (Distilled water as the solvent)	Increase in length of tops (centimeters)	Increase in length of roots (centimeters)
N/5 $SrCl_2$	0	0
N/7 $SrCl_2$	0.6	Slight
N/10 $SrCl_2$	0.6	Slight
N/500 $SrCl_2$	2.5	Slight, no lateral roots
N/1,000 $SrCl_2$	5.0	1.25 No lateral roots
N/2,000 $SrCl_2$	7.5	2.50 Lateral roots very short
N/5,000 $SrCl_2$	7.5	5.00 Lateral roots medium
Distilled water	7.5	6.25 Lateral roots medium

Little discussion of the results obtained with full nutrient and soil cultures is required, since the results, with the exception of strontium and barium, are so nearly uniform. The lethal concentrations of the various bases are practically identical, namely, N/3 to N/5. As a rule the seedlings withstand slightly stronger solutions when present in soil cultures. Of course, with longer duration of the experiments many concentrations that have not proved injurious would become so.

INFLUENCE OF PREVIOUS CONDITIONS OF GROWTH ON THE RESISTANCE TO THE TOXICITY OF VARIOUS IONS

Pea seedlings were grown for ten days in distilled water, full nutrient solution, and tap water, in order to determine whether age and the composition of the media influenced the resistance of seedlings to various ions. At the end of this period the seedlings were removed, carefully washed with distilled water, and placed in pure solutions of various salts. Final notes were taken three days later. In general it may be said that older seedlings are more resistant than young ones; for example, the roots of young seedlings are killed by N/50 NaCl, whereas the roots of older seedlings withstand much stronger solutions. The composition of the media has little influence on the resistance of the seedlings, as may be seen from the data summarized in Table 8.

TABLE 8. Influence of Previous Conditions on Toxicity

Distilled water	Full nutrient solution	Tap water
NaCl	NaCl	NaCl
N/10 dead N/15 dead N/20 wilting (slight growth) N/50 good	N/10 dead N/15 dying N/20 growing	N/10 dead N/15 dying N/20 growing
KCl	KCl	KCl
N/5 dead N/7 dead N/10 growing	N/5 dead N/7 dead (slight growth) N/10 growing	N/5 dead N/7 dead (slight growth) N/10 growing
NH_4Cl	NH_4Cl	NH_4Cl
N/20 dead N/40 dying N/70 growing	N/20 dead N/25 tips of leaves dying N/50 growing	N/10 dead N/25 growing—injured N/50 growing
$CaCl_2$	$CaCl_2$	$CaCl_2$
N/3 dead N/5 growing N/10 good	N/3 dead N/5 growing N/10 good	N/3 dead N/5 growing N/10 good
$MgCl_2$	$MgCl_2$	$MgCl_2$
N/10 dead N/20 dead N/50 good	N/10 dead N/20 dying N/40 growing	N/10 dead N/20 dying N/40 growing
$MnCl_2$	$MnCl_2$	$MnCl_2$
N/25 dead N/50 dead N/100 dying N/200 slight growth	N/25 dead N/50 tops dead, roots medium N/75 tops injured, roots medium N/100 tops injured, roots medium	N/25 dead N/75 tops dead, roots alive N/100 tops slight growth, dead; roots alive N/200 tops not injured
$BaCl_2$	$BaCl_2$	$BaCl_2$
N/100 dead N/300 dead N/500 slight growth	N/100 dead N/300 slight growth N/500 growing	N/100 dead N/200 dead N/300 slight growth N/500 growing

INFLUENCE OF CERTAIN SALTS ON GERMINATION OF CANADA FIELD PEA SEED

Experiments have been conducted also to determine the influence of various substances on the germination of pea seed. These experiments comprised two series: in the first series the effect of steeping the seed in pure solutions of the substances was determined; in the second, the effect of adding the solutions to a given soil was ascertained.

The procedure in the first series was as follows: Fifty uniform seeds were chosen and were steeped for twenty-four hours in 200 cubic centimeters of pure solutions of each substance to be studied. Following this treatment the seeds were removed, carefully rinsed in distilled water, and placed between filter papers over moistened sphagnum. When the roots of the control seed — those steeped in distilled water for twenty-four hours — had grown to about two inches in length final notes were taken.

The conditions for germination were identical, inasmuch as the treated seeds and the controls were placed in the same germinating pan. Ordinary room temperatures were maintained. The results obtained are summarized in the following table:

TABLE 9. The Effect of Certain Salts on Germination

Experiment with $CaCl_2$		
Steeped for twenty-four hours in following solution	Percentage of germination	Notes on condition of roots
Distilled water	96 to 98	Uniform germination. Roots 6.25 centimeters in length
N/1 $CaCl_2$	10	Roots about .6 centimeter in length, enlarged, tips dead
N/3 $CaCl_2$	95	Roots about 1.25 centimeter in length
N/5 $CaCl_2$	96	No injury

TABLE 9—(*continued*)

Experiment with $MgCl_2$

Steeped for twenty-four hours in following solution	Percentage of germination	Notes on condition of roots
Distilled water	96 to 98	Uniform germination. Roots 6.25 centimeters in length
N/1 $MgCl_2$	8	Roots .3 centimeter long, dead
N/3 $MgCl_2$	95	Great injury. Roots .3 to .6 centimeter long, curled
N/5 $MgCl_2$	96	Great injury. Roots 1.22 centimeter long, curled
N/10 $MgCl_2$	96	Slight injury. Roots 5 centimeters in length

Experiment with KCl

Steeped for twenty-four hours in following solution	Percentage of germination	Notes on condition of roots
Distilled water	96 to 98	Uniform germination. Roots 6.25 centimeters in length
N/1 KCl	5	Dead
N/3 KCl	96	Three roots are normal, remainder show great injury, 1.25 centimeter long
N/5 KCl	96	Great injury. Roots about 1.25 centimeter long
N/10 KCl	97	No injury

Experiment with NaCl

Steeped for twenty-four hours in following solution	Percentage of germination	Notes on condition of roots
Distilled water	96 to 98	Uniform germination. Roots 6.25 centimeters in length
N/1 NaCl	0	
N/3 NaCl	90	Great injury. Roots about .6 centimeter long
N/5 NaCl	95	Great injury. Roots about 1.25 centimeter long
N/10 NaCl	96	No appreciable injury

TABLE 9—*(concluded)*

Experiment with NH_4Cl

Steeped for twenty-four hours in following solution	Percentage of germination	Notes on condition of roots
Distilled water	96 to 98	Germination uniform. Roots 6.25 centimeters long
N/1 NH_4Cl	0	
N/3 NH_4Cl	0	
N/5 NH_4Cl	30	Roots about 1.25 centimeter long
N/10 NH_4Cl	75	Roots show great injury
N/20 NH_4Cl	80	Roots show slight injury

Experiment with $MnCl_2$

Steeped for twenty-four hours in following solution	Percentage of germination	Notes on condition of roots
Distilled water	96 to 98	Germination uniform. Roots 6.25 centimeters long
N/1 $MnCl_2$	6	Roots dead, barely emerged
N/3 $MnCl_2$	94	Great injury. Roots .6 to 1.25 centimeter long
N/5 $MnCl_2$	96	Great injury. Roots 1.25 centimeter long
N/10 $MnCl_2$	97	Two roots are about normal, remainder show great injury
N/50 $MnCl_2$	98	Twenty-five roots are about normal, remainder show injury

The ions of magnesium, sodium, potassium, and calcium are injurious in the order given, magnesium being only slightly more harmful than either sodium or potassium. Of the salts tested, NH_4Cl alone inhibits germination at N/3 concentration.

In general these results are similar to those obtained by Magowan (1908), who germinated wheat seeds in pure solutions of certain salts and allowed them to remain in the solutions for thirty days. It was found that magnesium chlorid, sodium chlorid, potassium chlorid, and calcium chlorid are toxic in the order given. The results obtained by Magowan are presented in the following table:

	.02 m	.04 m	.06 m	.08 m	.12 m	.18 m
$MgCl_2$	64mm	26mm	15mm	10mm	6mm	0mm
NaCl	446	321	210	134	54	24
KCl	462	333	220	144	63	31
$CaCl_2$	552	386	249	168	84	48

Ammonium and manganese ions are far more deleterious than the above. Seeds steeped in N/20 NH_4Cl show slight injury, while those steeped in N/50 $MnCl_2$ are greatly injured. Whether these variations are due to the difference in penetrability of the seed covering, the writer is unable to judge from the data that he has obtained.

The procedure in the second series of experiments was as follows: Two hundred and fifty grams of air-dry soil (volatile matter 3.45 per cent) was placed in glass tumblers. Ten uniform pea seeds were placed in each tumbler, about one inch below the surface of the soil. One hundred and twenty-five cubic centimeters of the solutions to be studied was added to each tumbler of soil. The chlorids of ammonium, magnesium, potassium, and calcium are injurious in the order given. These results suggest that fertilizers should be judiciously applied to soils.

TABLE 10. GERMINATION OF SEED IN SOIL AS AFFECTED BY SALTS

Experiment with $CaCl_2$

Composition of solution	Length of tops (centimeters)	Length of roots (centimeters)	Notes on condition of roots
Control	7.50	8.9 branched	
N/1 $CaCl_2$	0	0	
N/3 $CaCl_2$	0.60	1.25	Dead. Few lateral roots
N/5 $CaCl_2$	1.25	3.75	Dead. Few lateral roots
N/10 $CaCl_2$	7.50	7.00	No injury

TABLE 10—(*concluded*)

Experiment with $MgCl_2$

Composition of solution	Length of tops (centimeters)	Length of roots (centimeters)	Notes on condition of roots
N/3 $MgCl_2$	0	0	
N/5 $MgCl_2$	Slight	Slight	Lateral roots very short
N/10 $MgCl_2$	1.25	5.00	Few short lateral branches
N/20 $MgCl_2$	2.50	8.75	No injury

Experiment with KCl

Composition of solution	Length of tops (centimeters)	Length of roots (centimeters)	Notes on condition of roots
N/1 KCl	0	0	
N/3 KCl	0	0	
N/5 KCl	1.25	2.50	Great injury
N/10 KCl	1.25	5.00	Slight injury

Experiment with NH_4Cl

Composition of solution	Length of tops (centimeters)	Length of roots (centimeters)	Notes on condition of roots
N/3 NH_3Cl	0	0	
N/5 NH_4Cl	0	0	
N/10 NH_4Cl	1.85	5.00	Slight injury
N/20 NH_4Cl	2.50	5.25	No injury

CONCLUSIONS

Barium, strontium, ammonium, magnesium, sodium, and potassium, in the order given, when present in pure solution are very toxic to seedlings. This toxicity is greatly reduced by either full nutrient solutions or soil cultures.

Under the conditions of the experiments much stronger solutions are required in order to prevent top growth than to kill the roots of seedlings. Seedlings that have been grown for ten days in either distilled water, tap water, or full nutrient solutions are far more resistant to any toxicant studied than are those that are placed immediately in the toxic solutions.

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CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

VARIATION AND CORRELATION OF OATS (AVENA SATIVA)

PART I. STUDIES SHOWING THE EFFECT OF SEASONAL CHANGES ON BIOMETRICAL CONSTANTS

BY H. H. LOVE AND C. E. LEIGHTY
OF THE DEPARTMENT OF PLANT-BREEDING

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VARIATION AND CORRELATION OF OATS (AVENA SATIVA)

PART I. STUDIES SHOWING THE EFFECT OF SEASONAL CHANGES ON BIOMETRICAL CONSTANTS

VARIATION AND CORRELATION OF OATS (AVENA SATIVA)

PART I. STUDIES SHOWING THE EFFECT OF SEASONAL CHANGES ON BIOMETRICAL CONSTANTS[1]

(Received for publication April 9, 1914)

H. H. LOVE AND C. E. LEIGHTY[2]

Although considerable effort has been directed toward the breeding of oats, yet little is known concerning the variation and correlation of characters of this crop. Little evidence has been given to show what characters, if any, may be used as a basis for selection, or whether all characters are so variable and so affected by different seasons as to render impossible their use as a means of improvement. The same is true in regard to the correlation of characters. How are they affected by varying conditions from year to year, and is their relationship stable enough to be of importance in selection and improvement? From the study of the methods of oat-breeding, certain questions regarding the variation and correlation of the various characters naturally arise. Some of these are:

1. Do the tallest plants produce the most grain?
2. Do the tallest plants produce the largest seed?
3. As the number of culms increases, does the average yield per culm increase?
4. As the number of culms increases, does the average number of kernels produced by each culm increase or decrease?
5. As the number of culms increases, does the average weight of kernel increase or decrease?
6. Are these various relationships stable from year to year?

In an attempt to answer these questions it is the purpose of this paper to present evidence on the variation and correlation of the various characters of oats when the crops have been grown in different seasons, thereby coming under the influence of diverse environmental conditions.

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[1] Paper No. 49, Department of Plant-breeding, Cornell University, Ithaca, New York.

[2] In cooperation with the Bureau of Plant Industry, United States Department of Agriculture.

MATERIAL AND METHODS

Owing to the fact that the commercial varieties of grains are composed of a mixture of several types, and that as they are grown from year to year there may be a tendency toward a selective elimination of certain types, it was thought advisable to make this first study on a pure line. For this reason a pure line of Sixty Day oats was grown for the purpose.

The first year (1908) this pure line was grown in a drill row. It was consequently impossible to harvest the crop plant by plant. The entire row was harvested, and the studies were made on the individual culms.

In the years 1909, 1910, 1911, and 1912 the planting was done in a different manner. The soil was prepared and marked off in rows one foot apart, then the seeds were dropped about two inches apart in the rows. By this method it was possible to harvest the plants as individuals, so that the notes could be taken on each plant. The crops were all grown on the same type of soil with the exception of that of 1910, when it was necessary to use a different soil type. This was unfortunate, but unavoidable.

The crop of 1911 was so injured by storms that it was impossible to gather any statistical data for it.

The climatic conditions of 1910 were not so favorable as those of the other years. The month of June was very dry, followed by a dry July with high temperature. The table on the following page gives the amount of rainfall, hours of sunshine, and temperature for the seasons considered. From this table it is apparent that the average rainfall for the months of March, April, May, June, and July was much lower in 1910 than in any other of the years considered. This is important, as will be shown later by the behavior of the correlation constants.

At the time of harvesting, the individual plants were bound, and were thus preserved with all their culms intact. After being pulled they were well dried, and thereafter were handled carefully so that there would be as little shattering as possible.

The data for the years 1909, 1910, and 1912 used in this publication are: (1) Average height of plant, in centimeters, obtained by measuring separately each culm of a plant from the base of the culm to the base of the apical spikelet, and dividing the sum of these measurements by the number of culms making up the plant. (2) Total yield of plant,

TABLE 1. CLIMATIC CONDITIONS

	January	February	March	April	May	June	July
			Rainfall				
1908	2.27	2.47	2.24	3.85	4.30	1.26	4.75
1909	2.64	2.80	1.78	3.18	2.15	3.97	1.62
1910	3.07	3.31	0.30	3.66	4.20	1.14	1.62
1912	1.63	1.28	2.73	2.97	3.58	1.37	2.64
			Hours of sunshine				
1908	....	127	133	152	163	346	282
1909	86	84	165	197	231	298	337
1910	163	133	244	216	231	259	303
1912	129	178	182	172	280	359	337
			Temperature				
1908	25.2	20.8	35.0	44.2	58.8	67.6	72.7
1909	28.0	30.8	31.6	43.3	55.7	66.4	68.6
1910	25.3	23.2	42.0	49.8	54.2	63.8	71.4
1912	16.6	21.4	28.8	44.5	58.0	63.2	70.9

in grams, obtained by weighing the kernels produced. (3) Average yield of culm per plant, in grams, obtained by dividing the total yield by the number of culms of the plant. (4) Total number of kernels per plant. (5) Average number of kernels per culm per plant, obtained by dividing the total number of kernels by the number of culms of the plant. (6) Average weight of kernels per plant, in milligrams, obtained by dividing the total weight in grams of the kernels produced by their total number and expressing the result in milligrams. (7) Average number of spikelets per culm per plant, obtained by dividing the total number of spikelets by the number of culms of the plant. (8) Average number of kernels per spikelet per plant, obtained by dividing the total number of kernels produced by the plant by the total number of spikelets. (9) Number of culms per plant.

The data for 1908 are the actual determinations for single culms, as no averages for plants were possible because of the method used in taking the data. Items 2, 4, 8, and 9 in the preceding paragraph are not dealt with for that year, and the expression of the other items must be modified to suit the requirements.

After the data had all been taken, the values for the characters used in this study were placed on cards. The data for each individual were placed on a separate card, each card thus representing an individual. The cards were then sorted for the various frequency distributions and correlation tables. This method saves much time, since it is thus made possible to group the cards according to one character — such as average height of plant — and then, by keeping the different groups separate, to distribute them into correlation tables much more easily than two characters can be distributed into correlation tables from data not thus arranged.

For the first year, 1908, there were 825 individual culms used. For the years 1909, 1910, and 1912, plants were made the unit, as stated above, and there were 500, 400, and 400 used for the three years, respectively. The ordinary method for determining the mean, standard deviation, coefficient of correlation, and coefficient of variability was used. All determinations were made to the fourth decimal place and were recorded to the third. The mathematical work was doubly checked, and the authors feel that the constants are correct.

REVIEW OF LITERATURE

In recent years a number of biometrical studies have been made on the small grains by various investigators.

Waldron (1910)[3] reports biometrical results for oats and wheat, but the results for oats are discussed at length elsewhere in this paper and need not be considered here.

Love (1912), in a study of the question of large and small grain, reports biometrical results for a pure line of wheat. These results (Table 2) show that yield is associated with tall plants and with plants that produce a large number of kernels. At the same time the heaviest seed is produced in general on the tallest plants, on plants producing the largest number of seeds, and on the heaviest-yielding plants.

Myers (1912) made biometrical studies of a variety of wheat grown on plots of different fertility. He found correlations between: (1) number of culms and average weight of kernel, of .013±.032 on ordinary soil and .301±.027 on sand; (2) height and average weight of kernel, of .480±.025 on ordinary soil and .509±.022 on sand; (3) gross weight of

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[3] Dates in parenthesis refer to "Literature cited," page 70.

TABLE 2. CORRELATION COEFFICIENTS FOR THE DIFFERENT CHARACTERS OF WHEAT STUDIED

Characters	Correlation coefficients
Height of plant and yield	0.294 ± 0.032
Number of kernels per plant and yield	0.985 ± 0.001
Height of plant and average weight of grains	0.278 ± 0.033
Total number of grains per plant and average weight of grains	0.251 ± 0.033
Yield in grams and average weight of grains in milligrams	0.327 ± 0.031

culm and yield, of .925±.004 on ordinary soil and .890±.006 on manured soil. All correlations were greater on the poorer soil. Increased fertility tended to decrease variability.

Roberts (1912) made biometrical studies of three pure strains of wheat for two years. The characters considered were culm length, head length, number of culms per plant, number of grains per head, and yield. The means of the different characters are considerably modified by conditions of growth, increasing with improvement in these conditions, but a better growing season reduces their variability. A high positive correlation was found between yield and number of culms, and culm length and number of grains, and a fairly high positive correlation between culm length and head length, also between number of culms per plant and both culm length and number of grains. The correlation between culm length and head length varied considerably from one year to another, but there was little variation observed in the correlation between culm length and number of kernels.

The results reported by Leighty (1912) on a pure line of oats are incorporated in this paper and need not be discussed here.

Whitcomb (1913) reports biometrical results on two varieties of barley. The coefficients of correlation for average of plant, of the two varieties, are given in the table on the following page.

DEVELOPMENT AND VARIATION

The means, standard deviations, and coefficients of variability for the different characters under observation are given in Table 3. The mean

	New Zealand	Berkeley
Yield per culm and height of culm	.511±.029	.654±.022
Yield per culm and number of kernels per culm	.836±.012	.864±.010
Yield per culm and number of spikelets per culm	.965±.020	.521±.028
Yield per culm and average weight per kernel	.790±.015	.753±.017
Average weight per kernel and height of culm	.221±.037	.384±.033
Average weight per kernel and number of kernels per culm	.365±.034	.378±.033

for average height of plant in centimeters is 52.990±.181 for 1908. The plants were grown in drill rows, and the conditions were not so favorable for the development of tall culms in that year as they were in later years. The means for average height in the other years are, in order of value, 70.840±.234 for 1910, 75.200±.182 for 1912, and 76.110±.277 for 1909. The differences in height are not great for these years. The variability for this character as indicated by the standard deviation[4] is greatest for 1909 (the year for which the mean is largest), being then 9.188±.196; but the variability for 1908, 7.715±.128, is next in order although the mean is smallest for that year. This doubtless results from the fact that culms were then the units. For 1912 the variability is least, being then 5.405±.129; for 1910 it is 6.950±.166.

The means for the total yield of plant vary from 3.458±.045 grams for 1910 to 4.032±.068 for 1909 and 7.962±.113 for 1912. The variation as indicated by the standard deviations is in the same order, being 1.323±.032 for 1910, 2.249±.048 for 1909, and 3.353±.080 for 1912. The average yield of culm per plant has a mean for 1908 of .409±.006 gram, this being lower than the mean for any other year. For 1910 the mean is .962±.008, for 1909 it is .978±.010, and for 1912 it is 1.266±.008. The standard deviations for the average yield are

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[4] Variability is expressed by means of both the standard deviation and the coefficient of variability. The standard deviation expresses the variation as measured from the mean, and is expressed in the units of measurement. It is thus an absolute expression of variability. The coefficient of variability is a relative expression by means of which, for instance, a variable character whose data have been recorded in pounds is directly comparable with another whose data have been measured in inches. For the points under consideration the standard deviation very well expresses conditions as they actually are; at the same time the coefficient of variability is used also in these discussions.

TABLE 3. Means, Standard Deviations, and Coefficients of Variability for the Different Characters under Observation

	1908	1909	1910	1912
	M	M	M	M
Average height of plant in centimeters	52.990 ± .181	76.110 ± .277	70.840 ± .234	75.200 ± .182
Total yield of plant in grams		4.032 ± .068	3.458 ± .045	7.962 ± .113
Average yield of culm per plant in grams	.409 ± .006	.978 ± .010	.962 ± .008	1.266 ± .008
Total number of kernels per plant		247.500 ± 4.124	202.150 ± 2.657	548.650 ± 7.589
Average number of kernels per culm per plant	23.580 ± .327	59.800 ± .559	56.020 ± .484	86.600 ± .514
Average weight of kernels per plant in milligrams	16.114 ± .062	16.172 ± .040	17.100 ± .059	14.485 ± .036
Average number of spikelets per culm per plant	15.320 ± .173	32.340 ± .247	35.400 ± .245	
Average number of kernels per spikelet per plant		1.803 ± .007	1.506 ± .006	
Number of culms per plant		3.948 ± .043	3.568 ± .031	6.150 ± .066
	σ	σ	σ	σ
Average height of plant in centimeters	7.715 ± .128	9.188 ± .196	6.950 ± .166	5.405 ± .129
Total yield of plant in grams		2.249 ± .048	1.323 ± .032	3.353 ± .080
Average yield of culm per plant in grams	.257 ± .004	.315 ± .007	.241 ± .006	.239 ± .006
Total number of kernels per plant		136.560 ± 2.909	78.800 ± 1.879	225.050 ± 5.367
Average number of kernels per culm per plant	13.910 ± .231	18.508 ± .394	14.340 ± .342	15.250 ± .364
Average weight of kernels per plant in milligrams	2.626 ± .044	1.311 ± .028	1.741 ± .042	1.056 ± .025
Average number of spikelets per culm per plant	7.375 ± .122	8.171 ± .174	7.270 ± .173	
Average number of kernels per spikelet per plant		.229 ± .005	.172 ± .004	
Number of culms per plant		1.415 ± .030	.931 ± .022	1.972 ± .047
	C	C	C	C
Average height of plant in centimeters	14.559 ± .252	12.072 ± .261	9.811 ± .234	7.187 ± .171
Total yield of plant in grams		55.779 ± 1.514	38.259 ± 1.037	42.113 ± 1.169
Average yield of culm per plant in grams	62.836 ± 1.396	32.209 ± .754	25.052 ± .634	18.878 ± .466
Total number of kernels per plant		55.176 ± 1.490	38.981 ± 1.062	41.019 ± 1.131
Average number of kernels per culm per plant	58.991 ± 1.276	30.950 ± .720	25.598 ± .650	17.610 ± .433
Average weight of kernels per plant in milligrams	16.296 ± .278	8.106 ± .173	10.181 ± .245	7.290 ± .174
Average number of spikelets per culm per plant	48.140 ± .968	25.266 ± .572	20.537 ± .510	
Average number of kernels per spikelet per plant		12.701 ± .275	11.388 ± .275	
Number of culms per plant		35.841 ± .856	26.093 ± .663	32.065 ± .840

.239±.006 for 1912, .241±.006 for 1910, .257±.004 for 1908, and .315±.007 for 1909. The least variation exists where the mean is largest, but the decrease is not in proportion to the decrease in the mean.

In number of kernels the same conditions are found as obtained in yield. The means for total number of kernels are, in order of value, 202.150±2.657 for 1910, 247.500± 4.124 for 1909, and 548.650±7.589 for 1912. The means for average number per culm are, in order, 23.580±.327 (total number per culm) for 1908, 56.020±.484 for 1910, 59.800±.559 for 1909, and 86.600±.514 for 1912. In variability the order differs, being for total kernels 78.800±1.879 for 1910, 136.560±2.909 for 1909, and 225.050±5.367 for 1912; and for average number 13.910±.231 for 1908, 14.340±.342 for 1910, 15.250±.364 for 1912, and 18.508±.394 for 1909. The variability does not differ materially, but it is not greatest where the average yield is greatest.

The mean and the variability of average weight of kernels (milligrams in each case) are least for 1912, the mean being 14.485±.036 and the standard deviation 1.056±.025. The mean is greatest for 1910, being 17.100±.059, while the standard deviation for that year is 1.741±.042. The means for the other two years are about the same, being slightly above 16.1 in each case; the variation, however, is much greater for 1908, being then 2.626±.044, while for 1909 it is 1.311±.028.

The mean for number of spikelets per culm per plant is 15.320±.173 for 1908. This constant is again much smaller than the one for 1909, which is 32.340±.247, while the one for 1910 is 35.400±.245, the reason being undoubtedly the same as is given for the differences noted above. The variability is greatest for 1909, when the standard deviation is 8.171±.174. There is little difference in this respect between the other two years, the figures being 7.375±.122 for 1908 and 7.270±.173 for 1910.

The means for average number of kernels per spikelet were determined for only two years. For 1910 the mean is 1.506±.006, for 1909 it is 1.803±.007; while the standard deviations are .172±.004 and .229±.005, respectively. There is not much variability in this character, as is indicated by this constant.

The mean number of culms produced by the plant varies from 3.568±.031 for 1910 to 3.948±.043 for 1909, and 6.150±.066 for 1912, when it is greatest. The variability increases as does the mean, being

.931 ± .022 for 1910, 1.415 ± .030 for 1909, and 1.972 ± .047 for 1912. The number of culms produced by a plant is very responsive to environmental conditions. Under the best conditions here dealt with the number of culms has been largely increased, and the variability has also increased with the better conditions for growth.

The means for 1910 are less than those for 1909 and 1912 in average height, number of culms, total number and average number of kernels, total and average yield. When spikelet characters in 1909 and 1910 are compared, it is seen that for 1910 the mean is less for average number of kernels per spikelet and greater for average number of spikelets per culm than for 1909.

In regard to the comparative variability of the different characters, there is evidence in the coefficients of variability. From these it is observed that average height, average weight of kernels, and average number of kernels per spikelet, are the least variable characters, the coefficients of these ranging from about 7 to a little more than 16 per cent. In no other instance is the coefficient of variability less than 17, and from this it ranges to nearly 63 per cent.

The average weight of kernels per plant is higher for 1910 than for either of the other two years under consideration, but, since the total and the average number of kernels produced are less, the total and the average yields are still less for 1910. The average number of spikelets per culm per plant also is larger for 1910 than for 1909, but the average number of kernels per spikelet is larger for the latter year. It seems that when development is arrested by environmental conditions, yield is reduced by reduction in number of kernels per plant, per culm, and per spikelet, rather than in average weight of kernels and in number of spikelets produced. The actual kernel weights as given in the correlation tables show that larger kernels were actually produced in 1910 than in the other two years.

Altogether, then, the means indicate that the conditions for growth were not so favorable for the 1910 crop as for those of 1909 and 1912, and resulted in smaller plants and a reduced total yield of grain; but that these smaller plants produced a larger number of spikelets (compared with 1909 only) and larger kernels.

Somewhat different conditions are presented in the year 1908. In the first place, the data were taken on culms only — these being selected from a pure line grown in a drill row — and were taken on these selected culms without reference to the plants from which they came; while in the other years data are given for the entire plants and the culm is dealt with only in averages for the plants. But, by comparing the data for the culms of 1908 with the averages of the other years, it is seen that in 1908 the culms were not so tall as, and the number of spikelets, number of kernels, and yield were all less than, in the other years, but that the kernels were practically as large as, or larger than, in any other year except 1910. This condition offers evidence corroborative of that given above in regard to the relation of environment to variation.

Coupled with the less plant development and yield of 1908 and 1910, there is less variability, as indicated by the standard deviation, in number of culms, total and average number of kernels, total yield, average number of spikelets, and average number of kernels per spikelet. In average weight of kernels, however, there is greater variability for 1908 and 1910.

CORRELATION

The coefficients expressing the correlation between the different characters under observation are given in Table 4. These coefficients have been determined independently for the four years 1908, 1909, 1910, and 1912. The coefficients expressing the correlation between the same characters are directly comparable for the different years, except in so far as the results may be affected by (1) the use of culms as the unit of measurement in 1908 and of plants as the units in other years, and (2) the methods of growing the plants. The effect of the former has been shown to be small by a study of oats, by Dr. C. E. Leighty, soon to be published as Part II of this series. With reference to the methods of growing the plants, however, it is shown in the study just cited that rather large differences occur in the same variety between the coefficient of correlation determined for plants grown in hills and in drills.

By reference to Table 4 it is seen that the correlation between average height of plant and average weight of kernels per plant varies from .555 ± .016 for 1908 to —.023 ± .034 for 1910 — a considerable variation, which indicates that the correlation between these two characters is not stable nor constant, but fluctuates from year to year.

In contrast to this variation is the correlation between the total yield of plant and the total number of kernels per plant. In this case the coefficient for the different years varies from .980 ± .001 for 1912 to .918 ± .005 for 1910, a difference which is comparatively small.

TABLE 4. COEFFICIENTS OF CORRELATION BETWEEN DIFFERENT CHARACTERS UNDER OBSERVATION

	1908*	1909	1910	1912
Average height of plant in centimeters				
Total yield of plant in grams	....	.689 ± .016	.512 ± .025	.676 ± .018
Average yield of culm per plant in grams	.710 ± .012	.850 ± .008	.745 ± .015	.735 ± .016
Total number of kernels per plant	....	.676 ± .016	.487 ± .026	.662 ± .019
Average number of kernels per culm per plant	.658 ± .013	.835 ± .009	.732 ± .016	.730 ± .016
Average weight of kernels per plant in milligrams	.555 ± .016	.219 ± .029	—.023 ± .034	.217 ± .032
Average number of spikelets per culm per plant	.699 ± .012	.817 ± .010	.712 ± .017	....
Number of culms per plant	....	.413 ± .025	.042 ± .034	.523 ± .024
Total yield of plant in grams				
Average yield of culm per plant in grams	....	.761 ± .013	.694 ± .017	.692 ± .018
Total number of kernels per plant	.947 ± .002	.977 ± .001	.918 ± .005	.980 ± .001
Average number of kernels per culm per plant	....	.769 ± .012	.680 ± .018	.670 ± .019
Average weight of kernels per plant in milligrams	....	.149 ± .029	.035 ± .034	.220 ± .032
Average number of spikelets per culm per plant	.879 ± .005	.767 ± .012	.665 ± .019	....
Number of culms per plant	....	.850 ± .008	.712 ± .017	.912 ± .006
Average weight of kernels per plant in milligrams				
Average yield of culm per plant in grams	.464 ± .018	.337 ± .027	.225 ± .032	.429 ± .028
Total number of kernels per plant	....	.033 ± .030	—.253 ± .032	.071 ± .034
Average number of kernels per culm per plant	.300 ± .021	.121 ± .030	—.172 ± .033	.087 ± .033
Average number of spikelets per culm per plant	.329 ± .021	.120 ± .030	—.099 ± .033	....
Average number of kernels per spikelet per plant	....	.120 ± .030	—.190 ± .033	....
Number of culms per plant	....	—.021 ± .030	.184 ± .033	.050 ± .034
Average number of spikelets per culm per plant				
Average number of kernels per spikelet per plant	....	.324 ± .027	.253 ± .032	....
Number of kernels per culm	.880 ± .005	....	....	....
Number of culms per plant				
Average yield of culm per plant in grams	....	.387 ± .026	.070 ± .034	.383 ± .029
Average number of kernels per culm per plant	....	.423 ± 025	.132 ± .033	.408 ± .028

---

* The data for 1908 are, here and elsewhere, the total per culm, as these, not plants, were the units measured. "Average" and "per plant" should therefore be disregarded for this year.

From the two cases cited above and by an examination of other data contained in Table 4, it seems that correlation may be divided — with only fair accuracy, however — into two classes: (1) those which vary by considerable amount from year to year, and which may be known as fluctuating correlations; and (2) those which are fairly constant from year to year, and which may be known as stable correlations. The distinction between these two classes should be kept in mind in the following pages.

Correlation of average height of plant with other characters

The correlation between average height of plant and total yield of plant (Figs. 1, 2, 3), determined for three years, varies from .512 ± .025

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	
40–45	1														1
45–50	2	1													3
50–55	5	3													8
55–60	1	6	4												11
60–65	7	19	9					1							36
65–70	3	18	26	12	1										60
70–75	4	10	42	20	10	3	2	1		1	1				94
75–80	2	4	14	38	23	6	5	5						2	99
80–85			1	22	32	19	11	10	5			1	1		102
85–90		1		4	13	17	17	9	4	1	2				68
90–95					2	2	5	5	1		2	1			18
	25	62	96	96	81	47	40	31	10	2	5	2	1	2	500

FIG. 1.— *Average height of plant in centimeters, subject*
Total yield of plant in grams, relative. 1909
Coefficient of correlation = .689 ± .016

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	
45–50		1	1							2
50–55	1	6	2							9
55–60	2	6	7	5	1					21
60–65		13	9	9	3					34
65–70		7	46	28	15	1				97
70–75		13	23	43	32	10	2			123
75–80		4	13	20	22	27	2		1	89
80–85			5	3	7	4	3	2		24
85–90										0
90–95				1						1
	3	50	106	109	80	42	7	2	1	400

FIG. 2.— *Average height of plant in centimeters, subject*
Total yield of plant in grams, relative. 1910
Coefficient of correlation = .512 ± .025

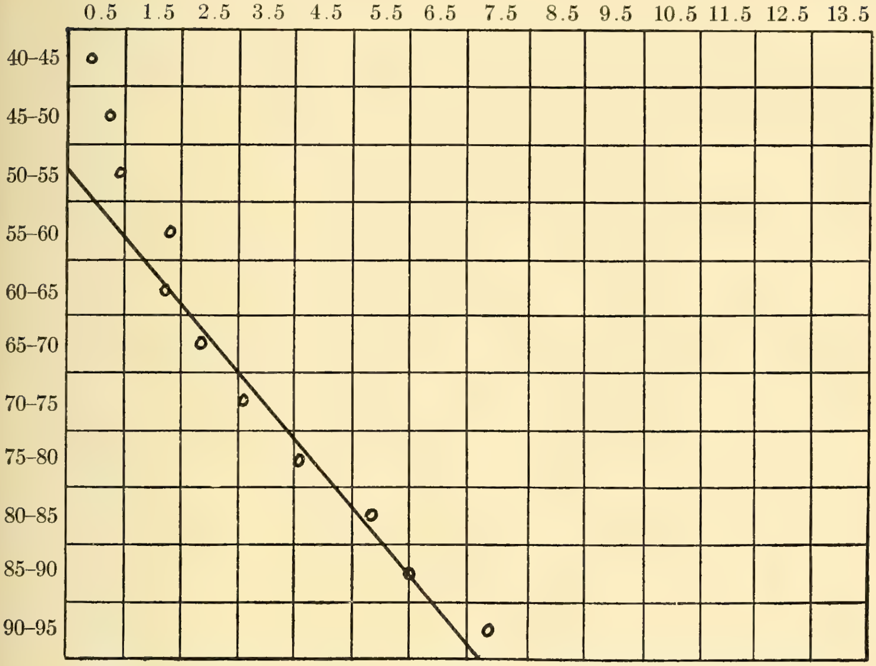

CHART 1.— *Regression for average height of plant and total yield for 1909, from Fig. 1*

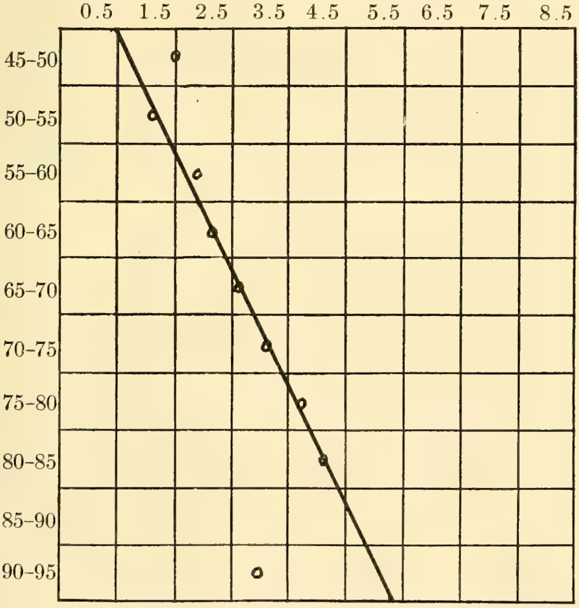

CHART 2.—*Regression for average height of plant and total yield for 1910, from Fig. 2*

	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	
55-60	1	1																		2
60-65		9	1	1																11
65-70		9	13	11	7	4	1													45
70-75	1	1	6	20	14	29	24	24	12	4	2		1	2						140
75-80			1	3	9	25	19	15	18	10	8	7	1	2	1		1	2		122
80-85				2		3	4	6	8	7	17	7	11	2	1	1		1	3	73
85-90									2	2	1		1	1						7
	2	20	21	37	30	61	48	45	40	23	28	14	14	7	2	1	1	3	3	400

FIG. 3.—*Average height of plant in centimeters, subject*
Total yield of plant in grams, relative. 1912
Coefficient of correlation =.676 ± .018

for 1910 to .689±.016 for 1909; while the correlation between average height of plant and average yield of culm per plant (Figs. 4, 5, 6, 7),

	0-.1	.1-.2	.2-.3	.3-.4	.4-.5	.5-.6	.6-.7	.7-.8	.8-.9	.9-1.0	1.0-1.1	1.1-1.2	1.2-1.3	1.3-1.4	1.4-1.5	1.5-1.6	1.6-1.7	1.7-1.8	
25-30	4																		4
30-35	6	1																	7
35-40	14	12	1																27
40-45	19	37	18	1	1														76
45-50	5	40	64	27	9	4	2												151
50-55	2	13	60	79	39	30	10	1	2					1					237
55-60		2	12	31	59	39	33	10	4		4	1				1			196
60-65		1	3	7	9	11	18	11	7	5	7		1	2		1			83
65-70			1	3	1	3	4	3	3	3	2	1		1				2	27
70-75		1	1	1		1	2	1		1	3	2	1		1				15
75-80								1		1									2
	50	107	160	149	118	88	69	27	16	10	16	4	2	4	1	2	0	2	825

FIG. 4.—*Height of culm in centimeters, subject*
Total yield of culm in grams, relative. 1908
Coefficient of correlation = .710 ± .012

	.1-.2	.2-.3	.3-.4	.4-.5	.5-.6	.6-.7	.7-.8	.8-.9	.9-1.0	1.0-1.1	1.1-1.2	1.2-1.3	1.3-1.4	1.4-1.5	1.5-1.6	1.6-1.7	1.7-1.8	1.8-1.9	
40-45	1																		1
45-50	1		1	1															3
50-55		2	4	2															8
55-60		1	2	1	7														11
60-65	1			8	9	9	6	3											36
65-70				2	11	9	26	8	3	1									60
70-75				2	6	13	18	20	17	11	5		1	1					94
75-80						1	6	18	32	21	14	4	1	2					99
80-85								4	12	20	24	22	8	8	2		2		102
85-90									1	4	11	12	17	14	3	3	3		68
90-95										1	1	1	3	2	5	2	2	1	18
	3	3	7	16	33	32	56	53	65	58	55	39	30	27	10	5	7	1	500

FIG. 5.— *Average height of plant in centimeters, subject*
Average yield of culm per plant in grams, relative. 1909
Coefficient of correlation = .850 ± .008

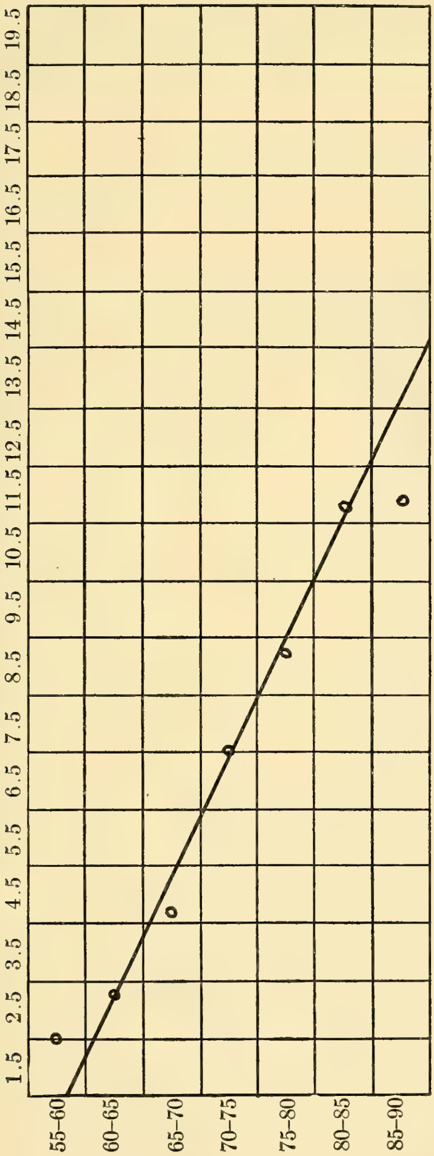

CHART 3.— *Regression for average height of plant and total yield for 1912, from Fig. 3*

	.3–.4	.4–.5	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	
45–50			1	1													2
50–55	3	2	4														9
55–60	1	2	4	8	2	2	1		1								21
60–65		2	6	6	10	5	3	1		1							34
65–70			3	11	21	27	15	10	8	2							97
70–75		1		2	5	23	31	34	17	8	2						123
75–80					3	3	15	16	18	20	11	3					89
80–85						1			5	4	7	5	1			1	24
85–90																	0
90–95													1				1
	4	7	18	28	41	61	65	61	49	35	20	8	2	0	0	1	400

Fig. 6.—*Average height of plant in centimeters, subject*
Average yield of culm per plant in grams, relative. 1910
Coefficient of correlation = .745 ± .015

	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	1.9–2.0	
55–60	1			1												2
60–65		4	3	4												11
65–70			2	11	16	4	9	3								45
70–75			1	5	6	17	35	43	19	10	2	2				140
75–80					2	4	18	32	25	20	9	9	3			122
80–85							3	8	11	20	12	13	4		2	73
85–90											4	1	1	1		7
	1	4	6	21	24	25	65	86	55	50	27	25	8	1	2	400

Fig. 7.—*Average height of plant in centimeters, subject*
Average yield of culm per plant in grams, relative. 1912
Coefficient of correlation = .735 ± .016

determined for four years, varies from .710 ± .012 for 1908 to .850 ± .008 for 1909. It is seen from this that the correlation coefficients in both cases are positive and high and that there is a marked degree of stability from year to year, but that there is at the same time some response to environment. There is a closer relation between culm yield and height than between total yield and height.

The regression lines have been determined for the average height of plant and total yield for the three years 1909, 1910, and 1912. These are shown in Charts 1, 2, and 3.

The correlation between average height of plant and total number of kernels per plant (Figs. 8, 9, 10), determined for three years, varies from .487 ± .026 for 1910 to .676 ± .016 for 1909; while the correlation between average height of plant and average number of kernels per culm per plant (Figs. 11, 12, 13, 14), determined for four years, varies from

	0– 50	50–100	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	500–550	550–600	600–650	650–700	700–750	750–800	
40–45	1																1
45–50	2	1															3
50–55	4	3	1														8
55–60	1	4	6														11
60–65	5	10	15	5						1							36
65–70	3	9	18	18	8	4											60
70–75	3	5	22	34	12	9	4	2	1			2					94
75–80	1	2	5	21	31	17	8	3	5	1	3				1	1	99
80–85			1	8	22	19	21	10	10	4	4	1			2		102
85–90		1		2	6	9	13	11	11	9	4		1	1			68
90–95					1		3	4	3	2	2		1	2			18
	20	35	68	88	80	58	49	30	30	17	13	3	2	3	3	1	500

FIG. 8.— *Average height of plant in centimeters, subject*
Total number of kernels per plant, relative. 1909
Coefficient of correlation = .676 ± .016

	0– 50	50–100	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	
45–50		1	1								2
50–55		3	4	2							9
55–60	2	4	8	3	4						21
60–65		6	13	8	5	2					34
65–70		3	30	32	21	9	2				97
70–75		4	25	29	34	19	7	5			123
75–80			11	17	13	25	19	2	1	1	89
80–85			3	4	4	3	5	1	3	1	24
85–90											0
90–95				1							1
	2	21	95	96	81	58	33	8	4	2	400

FIG. 9.— *Average height of plant in centimeters, subject*
Total number of kernels per plant, relative. 1910
Coefficient of correlation = .487 ± .026

	100– 150	150– 200	200– 250	250– 300	300– 350	350– 400	400– 450	450– 500	500– 550	550– 600	600– 650	650– 700	700– 750	750– 800	800– 850	850– 900	900– 950	950–1000	1000–1050	1050–1100	1100–1150	1150–1200	1200–1250	1250–1300	
55-60	2																								2
60-65		9	1	1																					11
65-70		7	7	9	8	3	6	5																	45
70-75	1	1	4	5	17	11	19	19	14	20	13	7	4	1	1	1	1	1							140
75-80			1		5	9	13	15	15	9	10	12	8	7	7	2	2	3			3	1			122
80-85				1	1	1	2	1	6	3	4	7	6	5	12	8	4	6		1	1	1	1	2	73
85-90											2	1	1	1			1	1							7
	3	17	13	16	31	24	40	40	35	32	29	27	19	14	20	11	8	11	0	1	4	2	1	2	400

FIG. 10.— *Average height of plant in centimeters, subject*
Total number of kernels per plant, relative. 1912
Coefficient of correlation = .662 ± .019

	1–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	
25–30	4										4
30–35	6	1									7
35–40	21	6									27
40–45	43	30	2	1							76
45–50	21	97	26	6		1					151
50–55	9	97	95	32	2	1			1		237
55–60	1	24	94	53	16	4	3		1		196
60–65	1	7	16	31	17	5	3	2	1		83
65–70		3	5	6	6	3		2		2	27
70–75		3		2	4	4	1	1			15
75–80				1	1						2
	106	268	238	132	46	18	7	5	3	2	825

FIG. 11.— *Height of culm in centimeters, subject*
Total number of kernels per culm, relative. *1908*
Coefficient of correlation = .658 ± .013

	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	
40–45	1											1
45–50	1	2										3
50–55	3	4	1									8
55–60	1	3	7									11
60–65		7	13	14	2							36
65–70		1	13	27	18			1				60
70–75		1	9	38	27	16	2	1				94
75–80			1	7	43	31	16	1				99
80–85				1	14	35	33	16	2	1		102
85–90					4	6	21	24	10	3		68
90–95							4	5	5	3	1	18
	6	18	44	87	108	88	76	48	17	7	1	500

FIG. 12.— *Average height of plant in centimeters, subject*
Average number of kernels per culm per plant, relative. *1909*
Coefficient of correlation = .835 ± .009

.658 ± .013 for 1908 to .835 ± .009 for 1909. In each case the correlation coefficients are positive, high, and fairly stable. This indicates that height and kernels produced depend on each other to a considerable extent, but that the relation may be modified by environmental conditions. There is a closer relation between height and kernel production for the average culm than for the whole plant.

	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	
45–50		2							2
50–55	5	3	1						9
55–60	6	8	5	1	1				21
60–65	2	9	18	4	1				34
65–70		9	41	33	12	2			97
70–75		2	28	45	34	13	1		123
75–80			3	26	27	24	9		89
80–85				1	2	12	6	3	24
85–90									0
90–95							1		1
	13	33	96	110	77	51	17	3	400

FIG. 13.— *Average height of plant in centimeters, subject*
Average number of kernels per culm per plant, relative. *1910*
Coefficient of correlation = *.732* ± *.016*

	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	120–130	
55–60	1		1								2
60–65		4	7								11
65–70			7	22	9	6	1				45
70–75			1	13	30	59	32	5			140
75–80				2	16	40	38	23	3		122
80–85					1	12	26	23	9	2	73
85–90								3	2	2	7
	1	4	16	37	56	117	97	54	14	4	400

FIG. 14.— *Average height of plant in centimeters, subject*
Average number of kernels per culm per plant, relative. *1912*
Coefficient of correlation = *.730* ± *.016*

The correlation between average height of plant and average weight of kernels per plant (Figs. 15, 16, 17, 18), determined for four years, varies from —.023±.034 for 1910 to .555±.016 for 1908. This correlation is fluctuating and ranges from what is virtually no correlation to a high correlation. From this it must be concluded that the relation between height of plant and average weight of kernels is occasionally close, but that it is responsive to, and is modified by, environment to such an extent that sometimes the two characters may be practically independent of each other.

	3.5– 4.5	4.5– 5.5	5.5– 6.5	6.5– 7.5	7.5– 8.5	8.5– 9.5	9.5–10.5	10.5–11.5	11.5–12.5	12.5–13.5	13.5–14.5	14.5–15.5	15.5–16.5	16.5–17.5	17.5–18.5	18.5–19.5	19.5–20.5	20.5–21.5	21.5–22.5	22.5–23.5	23.5–24.5	24.5–25.5	25.5–26.5	26.5–27.5	27.5–28.5	28.5–29.5	29.5–30.5	
25–30	1			1		1											1											4
30–35	1	1				1	2				2																	7
35–40		1			1		1		5	6	5	3	4	1														27
40–45					1		1	2	2	7	14	17	17	6	4	1	2	1				1						76
45–50							1	3	2	3	12	27	31	21	24	17	9						1					151
50–55											5	33	43	64	48	22	16	1	3	1	1							237
55–60								1				11	28	50	43	36	15	3	2	4	1			1			1	196
60–65				1								2	2	17	13	20	12	10	4	1	1							83
65–70													2	2	5	8	2	4	1	2	1							27
70–75								1					1	1	1	3	1	2	3	2								15
75–80																	1			1								2
	2	2	0	2	2	2	5	7	9	16	38	93	128	162	138	107	59	21	13	11	4	1	1	1	0	0	1	825

FIG. 15.—*Height of culm in centimeters, subject*
Average weight of kernels per culm in milligrams, relative. 1908
Coefficient of correlation = .555 ± .016

	8.5–9.5	9.5–10.5	10.5–11.5	11.5–12.5	12.5–13.5	13.5–14.5	14.5–15.5	15.5–16.5	16.5–17.5	17.5–18.5	18.5–19.5	19.5–20.5	
40–45								1					1
45–50			1				2						3
50–55						1	3	2	1	1			8
55–60						2	3	5	1				11
60–65	1	1			1	3	7	10	9	4			36
65–70				2	1	5	13	20	16	3			60
70–75	1		1			1	19	36	23	8	5		94
75–80						3	27	32	26	8	3		99
80–85						2	11	40	37	9	2	1	102
85–90				1		1	7	28	21	5	5		68
90–95					1		1	11	4		1		18
	2	1	2	3	3	18	93	185	138	38	16	1	500

FIG. 16.— *Average height of plant in centimeters, subject*
Average weight of kernels per plant in milligrams, relative. *1909*
Coefficient of correlation = *.219* ± *.029*

	11.5–12.5	12.5–13.5	13.5–14.5	14.5–15.5	15.5–16.5	16.5–17.5	17.5–18.5	18.5–19.5	19.5–20.5	20.5–21.5	21.5–22.5	22.5–23.5	23.5–24.5	
45–50							1	1						2
50–55		1	2	1	1	3			1					9
55–60			1	1	3	7	4	2	1	1	1			21
60–65		1	1	3	7	8	10	2		1	1			34
65–70		1	5	13	13	24	23	9	5	2	1		1	97
70–75		1	3	12	31	30	24	11	5	5	1			123
75–80	1		3	16	14	25	13	10	6	1				89
80–85			1	4	3	6	7	2	1					24
85–90														0
90–95							1							1
	1	4	16	50	72	103	83	37	19	10	4	0	1	400

FIG. 17.— *Average height of plant in centimeters, subject*
Average weight of kernels per plant in milligrams, relative. *1910*
Coefficient of correlation = *—.023* ± *.034*

	12	13	14	15	16	17	18	19	
55–60				2					2
60–65		2	5	3	1				11
65–70	1	12	22	8	2				45
70–75	3	27	52	43	10	2	2	1	140
75–80		11	50	42	15	3		1	122
80–85	1	2	25	28	14	2	1		73
85–90			4	2		1			7
	5	54	158	128	42	8	3	2	400

FIG. 18.— *Average height of plant in centimeters, subject*
Average weight of kernels per plant in milligrams, relative. *1912*
Coefficient of correlation = *.217* ± *.032*

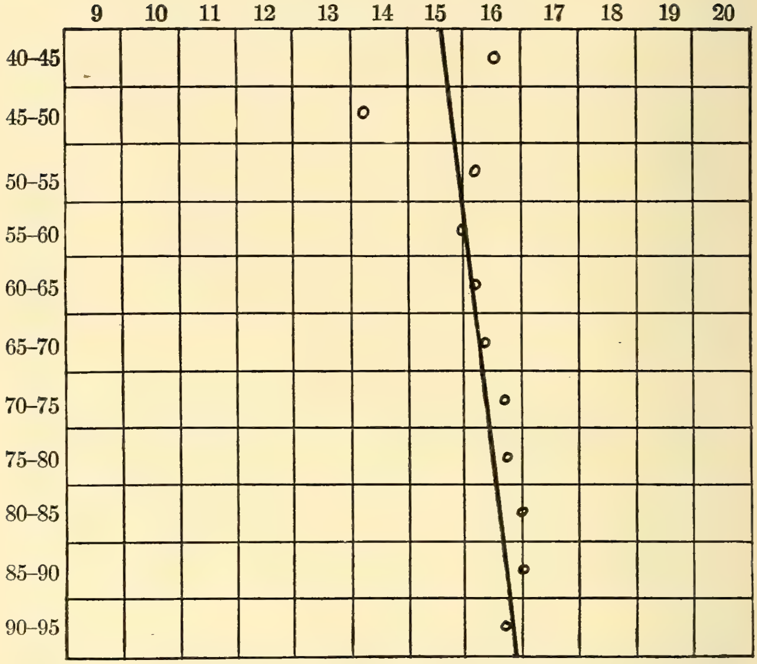

Chart 4.— *Regression for average height of plant and average weight of kernels for 1909, from Fig. 16*

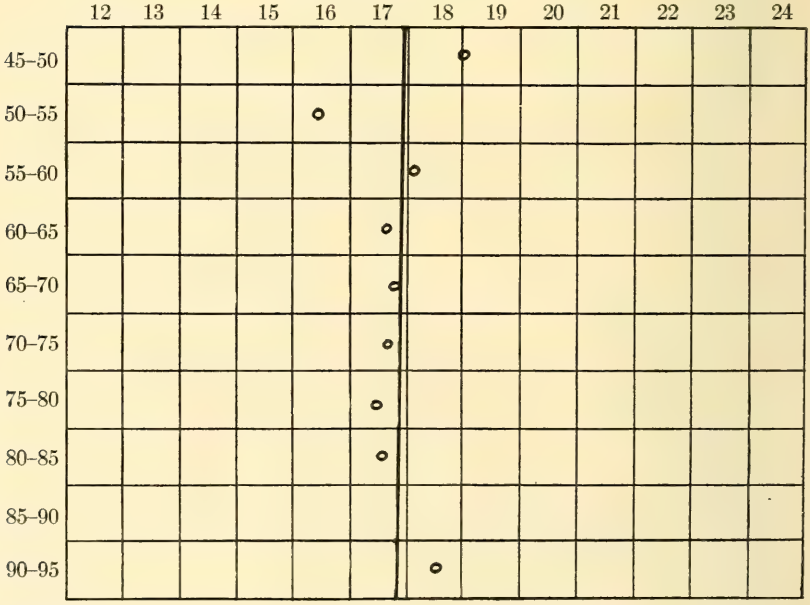

Chart 5.—*Regression for average height of plant and average weight of kernels for 1910, from Fig. 17*

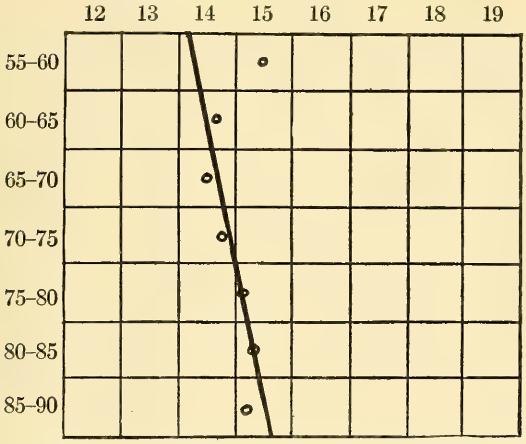

CHART 6.— *Regression for average height of plant and average weight of kernels for 1912, from Fig. 18*

The regression lines have been determined for the average height of plant and average weight of kernels for the three years 1909, 1910, and 1912. These are shown in Charts 4, 5, and 6.

The correlation between average height of plant and average number of spikelets per culm per plant (Figs. 19, 20, 21), determined for three

	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	
25–30	3	1									4
30–35	5	1	1								7
35–40	9	15	2	1							27
40–45	13	50	11	2							76
45–50	3	74	55	15	3		1				151
50–55		35	101	66	30	4		1			237
55–60		9	36	76	48	21	4	2			196
60–65		1	7	18	30	18	5	4			83
65–70			2	7	5	7	3		2	1	27
70–75				2	5	6	1		1		15
75–80					2						2
	33	186	215	187	123	56	14	7	3	1	825

FIG. 19.— *Height of culm in centimeters, subject*
Number of spikelets per culm, relative. 1908
Coefficient of correlation = .699 ± .012

	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	
40–45	1									1
45–50	1	1	1							3
50–55	2	5	1							8
55–60	2	4	5							11
60–65	2	10	16	6	2					36
65–70		7	10	34	8	1				60
70–75		1	18	37	27	9	2			94
75–80			6	16	43	29	5			99
80–85				6	29	42	20	5		102
85–90				1	6	20	29	11	1	68
90–95						1	5	10	2	18
	8	28	57	100	115	102	61	26	3	500

Fig. 20.— *Average height of plant in centimeters, subject*
Average number of spikelets per culm per plant, relative. 1909
Coefficient of correlation = .817 ± .010

	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	
45–50		1	1						2
50–55	3	5	1						9
55–60	4	8	5	2		2			21
60–65	2	5	15	9	3				34
65–70		2	26	37	24	5	3		97
70–75			11	37	48	23	4		123
75–80				11	35	27	12	4	89
80–85				1	2	9	8	4	24
85–90									0
90–95							1		1
	9	21	59	97	112	66	28	8	400

Fig. 21.— *Average height of plant in centimeters, subject*
Average number of spikelets per culm per plant, relative. 1910
Coefficient of correlation = .712 ± .017

years, varies from .699 ± .012 for 1908 to .817 ± .010 for 1909. The correlation is fairly stable and very high. From this it may be concluded that the number of spikelets produced is largely dependent on the vigor of the plant as expressed in height of culm, the taller plants tending very strongly to produce large numbers of spikelets.

The correlation between average height of plant and number of culms per plant (Figs. 22, 23, 24), determined for three years, varies from

	1	2	3	4	5	6	7	8	9	10	11	
40–45		1										1
45–50	1	1	1									3
50–55		5		3								8
55–60		2	3	5	1							11
60–65	3	13	11	8					1			36
65–70	3	8	27	15	7							60
70–75	3	12	29	35	8	3	3	1				94
75–80	2	6	22	45	10	3	7	2	1		1	99
80–85		2	15	48	16	13	6	2				102
85–90	1		6	31	17	11	2					68
90–95			1	8	5	1	1	2				18
	13	50	115	198	64	31	19	7	2	0	1	500

FIG. 22.— *Average height of plant in centimeters, subject Number of culms per plant, relative. 1909 Coefficient of correlation = .413 ± .025*

	2	3	4	5	6	7	
45–50		1	1				2
50–55	2	3	3			1	9
55–60	4	9	4	2	2		21
60–65	6	10	13	4	1		34
65–70	8	40	42	6	1		97
70–75	15	41	53	12	2		123
75–80	10	22	43	11	3		89
80–85	4	8	8	3	1		24
85–90							0
90–95	1						1
	50	134	167	38	10	1	400

FIG. 23.— *Average height of plant in centimeters, subject Number of culms per plant, relative. 1910 Coefficient of correlation = .042 ± .034*

	2	3	4	5	6	7	8	9	10	11	12	13	14	
55–60	1		1											2
60–65		6	3	2										11
65–70	1	10	17	9	6	2								45
70–75	2	5	24	32	31	26	15	3	2					140
75–80		2	14	29	15	18	32	7	1	3			1	122
80–85		2	2	6	5	14	22	12	6	2	2			73
85–90					4	1	1	1						7
	4	25	61	78	61	61	70	23	9	5	2	0	1	400

FIG. 24.— *Average height of plant in centimeters, subject Number of culms per plant, relative. 1912 Coefficient of correlation = .523 ± .024*

.042±.034 for 1910 to .523±.024 for 1912. This correlation, like the one just considered, is fluctuating and ranges from no correlation to a high correlation. And in the same way it must be concluded that the correlation which exists between the height of plant and the number of culms produced by that plant is strong under some conditions, but that it is responsive to, and is modified by, environment to such an extent that on occasion the two characters may be practically independent of each other.

Considering the relation of average height of plant to the other plant characters as a whole, it has been shown that there is generally a very high, positive, and stable correlation between the average height of plant and (1) total and average yield, (2) total and average number of kernels produced, (3) average number of spikelets per culm. These relations are disturbed to some extent by environmental conditions such as exist in different years. It is conceivable that they might vary to a greater extent, even to such a degree that there would be no correlation between these characters. Under very abnormal weather conditions which would allow vegetative development to some extent but under which grain development would be prohibited, correlations would be impossible; but correlations such as those mentioned above are likely to obtain in any case when kernels are produced in sufficient quantity to render possible the determination of averages. Whenever kernels are developed, the taller plants would tend to develop the greater numbers of spikelets and kernels, and consequently to produce the higher yields.

The correlation of average height with the averages per culm are higher than those with the plant totals. This is undoubtedly due to the fact that height itself is an average, and when averages are used there has been effected a similar and simultaneous change in both the height and the other character under consideration.

The correlations between height of plant and (1) average weight of kernels, (2) number of culms, are fluctuating in amount, occasionally being high, but being influenced by environmental conditions from year to year so that the relation of one to the other may become such that they vary independently. A short plant, then, on occasion may develop as large kernels as a tall plant, or may develop as many culms.

Correlation of total yield of plant with other characters

The correlation between total yield of plant and average yield of culm per plant (Figs. 25, 26, 27), determined for three years, varies from .692±.018 for 1912 to .761±.013 for 1909. This correlation is very high, positive, and stable—as would be expected, in fact. It is not by any means perfect correlation, however, this being due to the method of development in the oat plant. All culms do not develop equally,

	.1–.2	.2–.3	.3–.4	.4–.5	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	
0–1	3	3	3	6	1	3	3	3											25
1–2			4	10	22	8	7	5	5								1		62
2–3					10	18	28	20	12	4	2		1	1					96
3–4						3	15	17	29	14	12	5					1		96
4–5							3	4	12	27	22	7		4	2				81
5–6									2	6	7	11	11	10					47
6–7								3	4		5	7	10		5	3	3		40
7–8								1	1	6	5	8	1	5	3			1	31
8–9										1		1	4	3		1			10
9–10											1		1						2
10–11													1	2		1	1		5
11–12													1	1					2
12–13																	1		1
13–14											1			1					2
	3	3	7	16	33	32	56	53	65	58	55	39	30	27	10	5	7	1	500

FIG. 25.— *Total yield of plant in grams, subject*
Average yield of culm per plant in grams, relative. 1909
Coefficient of correlation = .761 ± .013

	.3–.4	.4–.5	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	
0–1	1	1					1										3
1–2	2	5	13	9	5	6	10										50
2–3	1	1	5	13	25	25	18	6	5	4	2	1					106
3–4				5	9	24	28	16	18	7	1		1				109
4–5				1	2	6	6	31	17	10	3	3	1				80
5–6							2	7	7	11	13	2					42
6–7								1	2	3	1						7
7–8												1				1	2
8–9												1					1
	4	7	18	28	41	61	65	61	49	35	20	8	2	0	0	1	400

FIG. 26.— *Total yield of plant in grams, subject*
Average yield of culm per plant in grams, relative. 1910
Coefficient of correlation = .694 ± .017

	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	1.9–2.0	
1– 2				2												2
2– 3	1	3	4	9	1			2								20
3– 4		1	2	4	7	3	3	1								21
4– 5				3	6	8	11	5	2	1		1				37
5– 6					4	1	13	5	2	5						30
6– 7				1	4	4	10	20	13		5	3	1			61
7– 8				2	2	6	5	13	3	12	5					48
8– 9						3	12	11	8	7		3	1			45
9–10							8	13	12	1	4	2				40
10–11							1	9	6	4	2		1			23
11–12							1	5	5	12		4		1		28
12–13									1	5	5	3				14
13–14								1	3	2	3	4			1	14
14–15								1		1	2	2	1			7
15–16							1				1					2
16–17												1				1
17–18													1			1
18–19												1	2			3
19–20												1	1		1	3
	1	4	6	21	24	25	65	86	55	50	27	25	8	1	2	400

Fig. 27.— *Total yield of plant in grams, subject*
Average yield of culm per plant in grams, relative. 1912
Coefficient of correlation = .692 ± .018

	1– 10	10– 20	20– 30	30– 40	40– 50	50– 60	60– 70	70– 80	80– 90	90–100	
*0– .1	50										50
.1– .2	54	53									107
.2– .3	2	154	3		1						160
.3– .4		56	93								149
.4– .5		5	109	4							118
.5– .6			29	55	2	2					88
.6– .7			4	59	6						69
.7– .8				11	16						27
.8– .9				3	11	2					16
.9–1.0					6	4					10
1.0–1.1					3	7	5	1			16
1.1–1.2					1	2	1				4
1.2–1.3						1	1				2
1.3–1.4								3	1		4
1.4–1.5								1			1
1.5–1.6									2		2
1.6–1.7											0
1.7–1.8										2	2
	106	268	238	132	46	18	7	5	3	2	825

Fig. 28.— *Total yield of culm in grams, subject*
Total number of kernels per culm, relative. 1908
Coefficient of correlation = .947 ± .002

* Since, as before noted, the data were taken on culms this year, the classes for yield differ from those of the other years.

some being much less important than others, and the average yield of those making up a plant is therefore not related absolutely to the total yield of the plant.

The correlation between total yield of plant and total number of kernels per plant (Figs. 28, 29, 30, 31), determined for four years, varies from

	0–50	50–100	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	500–550	550–600	600–650	650–700	700–750	750–800	
0– 1	20	4	1														25
1– 2		31	30	1													62
2– 3			37	58	1												96
3– 4				29	59	8											96
4– 5					20	47	14										81
5– 6						3	34	9		1							47
6– 7							1	21	18								40
7– 8									12	15	4						31
8– 9										1	8	1					10
9–10											1	1					2
10–11												1	2	2			5
11–12														1	1		2
12–13															1		1
13–14															1	1	2
	20	35	68	88	80	58	49	30	30	17	13	3	2	3	3	1	500

FIG. 29.— *Total yield of plant in grams, subject*
Total number of kernels per plant, relative. 1909
Coefficient of correlation = .977 ± .001

	0–50	50–100	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	
0–1	2	1									3
1–2		20	30								50
2–3			61	45							106
3–4			4	47	51	7					109
4–5				4	30	39	7				80
5–6						12	26	4			42
6–7								3	4		7
7–8								1		1	2
8–9										1	1
	2	21	95	96	81	58	33	8	4	2	400

FIG. 30.— *Total yield of plant in grams, subject*
Total number of kernels per plant, relative. 1910
Coefficient of correlation = .918 ± .005

	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	500–550	550–600	600–650	650–700	700–750	750–800	800–850	850–900	900–950	950–1000	1000–1050	1050–1100	1100–1150	1150–1200	1200–1250	1250–1300	
1–2	2																								2
2–3	1	17	2																						20
3–4			11	10																					21
4–5				6	25	5	1																		37
5–6					5	13	11	1																	30
6–7					1	5	26	25	4																61
7–8						1	2	14	18	10	3														48
8–9									12	19	13	1													45
9–10									1	3	11	18	6	1											40
10–11											2	7	8	6											23
11–12												1	5	6	13	2		1							28
12–13														1	6	5	1	1							14
13–14															1	3	4	6							14
14–15																1	3	3							7
15–16																				1	1				2
16–17																					1				1
17–18																					1				1
18–19																					1	1	1		3
19–20																						1		2	3
	3	17	13	16	31	24	40	40	35	32	29	27	19	14	20	11	8	11	0	1	4	2	1	2	400

FIG. 31.— *Total yield of plant in grams, subject*
Total number of kernels per plant, relative. 1912
Coefficient of correlation = .980 ± .001

.918±.005 for 1910 to .980±.001 for 1912; while that between total yield and average number of kernels per culm per plant (Figs. 32, 33, 34), determined for three years, varies from .670±.019 for 1912 to .769±.012 for 1909. These correlations are very high, especially those between the totals, and are stable from year to year. In fact, since the total

	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	
0–1	6	7	3	8	1							25
1–2		11	27	15	6	2			1			62
2–3			14	41	30	9	2					96
3–4				19	45	22	9	1				96
4–5				2	19	37	15	8				81
5–6					2	9	15	18	3			47
6–7				1	4	4	13	8	7	3		40
7–8				1	1	5	15	3	5		1	31
8–9							2	7		1		10
9–10							2					2
10–11							1	1	1	2		5
11–12								2				2
12–13										1		1
13–14							2					2
	6	18	44	87	108	88	76	48	17	7	1	500

FIG. 32.— *Total yield of plant in grams, subject*
Average number of kernels per culm per plant, relative. *1909*
Coefficient of correlation = .769 ± .012

	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	
0–1	3								3
1–2	7	17	13	9	3	1			50
2–3	2	13	47	31	8	3	2		106
3–4	1	2	27	45	29	4	1		109
4–5		1	9	19	28	20	2	1	80
5–6				6	8	18	10		42
6–7					1	4	2		7
7–8								2	2
8–9						1			1
	13	33	96	110	77	51	17	3	400

FIG. 33.— *Total yield of plant in grams, subject*
Average number of kernels per culm per plant, relative. *1910*
Coefficient of correlation = .680 ± .018

	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	120–130	
1– 2			1	1							2
2– 3	1	3	11	3	1	1					20
3– 4		1	2	12	4	2					21
4– 5			2	5	13	11	4	2			37
5– 6				4	8	12	4	2			30
6– 7				6	10	22	18	4	1		61
7– 8				4	7	18	12	7			48
8– 9				1	9	15	15	5			45
9–10					3	19	14	3	1		40
10–11				1		7	11	3	1		23
11–12						6	9	10	2	1	28
12–13						1	5	6	2		14
13–14						2	3	5	1	3	14
14–15						1	2	3	1		7
15–16					1			1			2
16–17									1		1
17–18									1		1
18–19								1	2		3
19–20								2	1		3
	1	4	16	37	56	117	97	54	14	4	400

FIG. 34.— *Total yield of plant in grams, subject*
Average number of kernels per culm per plant, relative. *1912*
Coefficient of correlation = .670 ± .019

	8.5– 9.5	9.5–10.5	10.5–11.5	11.5–12.5	12.5–13.5	13.5–14.5	14.5–15.5	15.5–16.5	16.5–17.5	17.5–18.5	18.5–19.5	19.5–20.5	
0– 1	1	1	1			1	3	9	6	3			25
1– 2	1			1		6	13	20	18	2	1		62
2– 3			1	1	2	3	26	36	17	8	2		96
3– 4						5	17	32	31	5	5	1	96
4– 5					1	2	12	27	21	13	5		81
5– 6				1			7	17	20	2			47
6– 7						1	8	17	9	3	2		40
7– 8							5	16	9	1			31
8– 9							1	6	3				10
9–10								1	1				2
10–11							1	2	1		1		5
11–12								2					2
12–13									1				1
13–14									1	1			2
	2	1	2	3	3	18	93	185	138	38	16	1	500

FIG. 35.— *Total yield of plant in grams, subject*
Average weight of kernels per plant in milligrams, relative. *1909*
Coefficient of correlation = .149 ± .029

	11.5–12.5	12.5–13.5	13.5–14.5	14.5–15.5	15.5–16.5	16.5–17.5	17.5–18.5	18.5–19.5	19.5–20.5	20.5–21.5	21.5–22.5	22.5–23.5	23.5–24.5	
0–1			1			1	1							3
1–2	1	3	2	5	12	13	10	2	2					50
2–3			5	13	14	31	23	11	6	2	1			106
3–4		1	4	9	23	20	26	13	6	5	2			109
4–5			2	15	15	18	16	6	3	3	1		1	80
5–6			1	6	5	18	7	4	1					42
6–7			1	1	3	2								7
7–8				1					1					2
8–9								1						1
	1	4	16	50	72	103	83	37	19	10	4	0	1	400

FIG. 36.— *Total yield of plant in grams, subject*
Average weight of kernels per plant in milligrams, relative. *1910*
Coefficient of correlation = .035 ± .034

	11.5–12.5	12.5–13.5	13.5–14.5	14.5–15.5	15.5–16.5	16.5–17.5	17.5–18.5	18.5–19.5	
1– 2			1	1					2
2– 3		4	11	4	1				20
3– 4		7	6	7	1				21
4– 5	2	10	15	10					37
5– 6	2	2	10	11	4		1		30
6– 7		5	29	15	9	2		1	61
7– 8		11	16	14	5		1	1	48
8– 9		5	18	14	8				45
9–10		6	17	12	3	1	1		40
10–11		1	10	10		2			23
11–12	1	2	13	7	4	1			28
12–13		1	4	7	2				14
13–14			6	6	2				14
14–15				6	1				7
15–16			2						2
16–17				1					1
17–18				1					1
18–19				1	1	1			3
19–20				1	1	1			3
	5	54	158	128	42	8	3	2	400

FIG. 37.— *Total yield of plant in grams, subject*
Average weight of kernels per plant in milligrams, relative. *1912*
Coefficient of correlation = .220 ± .032

yield is made up of the total number of kernels, such a condition is to be expected unless excessive variation in size of kernels should exist; which is not the case, as kernel weight is one of the least variable characters. The higher correlation of total yield with total number of kernels than with average number of kernels per culm results from the process of averaging, since there are a number of low-producing culms whose results affect the average yield per culm for many of the plants.

The correlation between total yield of plant and average weight of kernels per plant (Figs. 35, 36, 37), determined for three years, varies from .035±.034 for 1910 to .220±.032 for 1912. This correlation is fluctuating, and never very high, which indicates that large kernels are obtained from plants either low or high in yield in about the same proportions. In other words, the kernels produced by plants whether of large or small yield do not vary considerably in average size.

The correlation between total yield of plant and average number of spikelets per culm per plant (Figs. 38, 39, 40), determined for three years,

	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	
*0– .1	25	21	4								50
.1– .2	8	83	10	4	2						107
.2– .3		72	78	4	6						160
.3– .4		9	94	40	5	1					149
.4– .5		1	26	82	8	1					118
.5– .6			3	40	40	4	1				88
.6– .7				15	39	15					69
.7– .8				1	11	14	1				27
.8– .9				1	6	6	3				16
.9–1.0					3	6	1				10
1.0–1.1					2	6	6	2			16
1.1–1.2					1	1	1	1			4
1.2–1.3						1	1				2
1.3–1.4						1		2	1		4
1.4–1.5									1		1
1.5–1.6								2			2
1.6–1.7											0
1.7–1.8									1	1	2
	33	186	215	187	123	56	14	7	3	1	825

FIG. 38.— *Total yield of culm in grams, subject*
Average number of spikelets per culm, relative. 1908
Coefficient of correlation = .879 ± .005

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*Since, as before noted, the data were taken on culms this year, the classes for yield differ from those of the other years.

	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	
0– 1	7	7	7	3	1					25
1– 2	1	19	23	15	2	1	1			62
2– 3		2	23	45	23	2	1			96
3– 4			3	31	44	16	2			96
4– 5				4	30	34	10	3		81
5– 6			1		4	20	19	3		47
6– 7				2	6	9	16	6	1	40
7– 8					5	15	4	6	1	31
8– 9						3	4	3		10
9–10							2			2
10–11						1	1	2	1	5
11–12								2		2
12–13								1		1
13–14						1	1			2
	8	28	57	100	115	102	61	26	3	500

FIG. 39.— *Total yield of plant in grams, subject*
Average number of spikelets per culm per plant, relative. 1909
Coefficient of correlation = .767 ± .012

	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	
0–1	3								3
1–2	4	13	14	11	6	1		1	50
2–3	2	7	30	40	19	6	2		106
3–4		1	13	31	45	16	3		109
4–5			2	15	33	20	9	1	80
5–6					9	18	11	4	42
6–7						4	2	1	7
7–8							1	1	2
8–9						1			1
	9	21	59	97	112	66	28	8	400

FIG. 40.— *Total yield of plant in grams, subject*
Average number of spikelets per culm per plant, relative. 1910
Coefficient of correlation = .665 ± .019

varies from .665 ± .019 for 1910 to .879 ± .005 for 1908. This correlation is very high, positive, and fairly stable. It indicates that the total yield is dependent to a large extent on the number of spikelets produced. The correlation of yield and total number of spikelets would undoubtedly be higher than that with average number of spikelets per culm.

The correlation between total yield of plant and number of culms per plant (Figs. 41, 42, 43), determined for three years, varies from .712±.017 for 1910 to .912±.006 for 1912. This is positive, very high, and fairly stable. It indicates that as the number of culms per plant increases there is a very marked increase in the total yield of grain, or that the total yield of grain is highly responsive to the culm production of the plant.

	1	2	3	4	5	6	7	8	9	10	11	
0- 1	12	13										25
1- 2	1	28	24	8	1							62
2- 3		8	54	34								96
3- 4		1	31	56	8							96
4- 5			6	56	16	3						81
5- 6				32	13	2						47
6- 7				11	17	5	6	1				40
7- 8				1	8	12	8	1	1			31
8- 9					1	7	1	1				10
9-10							1	1				2
10-11						2	2	1				5
11-12								2				2
12-13							1					1
13-14									1		1	2
	13	50	115	198	64	31	19	7	2	0	1	500

FIG. 41.—*Total yield of plant in grams, subject*
Number of culms per plant, relative. 1909
Coefficient of correlation = .850 ± .008

	2	3	4	5	6	7	
0-1	3						3
1-2	28	19	3				50
2-3	18	66	20	1		1	106
3-4	1	42	58	7	1		109
4-5		7	59	11	3		80
5-6			26	14	2		42
6-7				4	3		7
7-8			1	1			2
8-9					1		1
	50	134	167	38	10	1	400

FIG. 42.—*Total yield of plant in grams, subject*
Number of culms per plant, relative. 1910
Coefficient of correlation = .712 ± .017

	2	3	4	5	6	7	8	9	10	11	12	13	14	
1- 2	2													2
2- 3	2	14	4											20
3- 4		7	12	2										21
4- 5		4	24	8	1									37
5- 6			12	14	4									30
6- 7			9	33	15	4								61
7- 8				17	18	9	4							48
8- 9				4	15	22	4							45
9-10					6	15	19							40
10-11					1	6	15	1						23
11-12					1	4	15	7	1					28
12-13							8	6						14
13-14						1	4	5	3	1				14
14-15							1	4	1		1			7
15-16									1				1	2
16-17									1					1
17-18									1					1
18-19										3				3
19-20									1	1	1			3
	4	25	61	78	61	61	70	23	9	5	2	0	1	400

FIG. 43.— *Total yield of plant in grams, subject*
Number of culms per plant, relative. 1912
Coefficient of correlation = .912 ± .006

Considering the relation of total yield of plant to other plant characters, it must be concluded that increase in yield must be accompanied by increase in (1) culm yield, (2) kernel production, both total and average, (3) average spikelet production, and (4) culm production; while, on the other hand, the average weight of kernels is practically independent of yield.

Correlation of average weight of kernels per plant with other characters

The correlation between average weight of kernels per plant and average yield of culm per plant (Figs. 44, 45, 46, 47), determined for four years, varies from .225 ± .032 for 1910 to .464 ± .018 for 1908. This correlation is not very high and fluctuates considerably. It is higher, however, than that between the average weight of kernels and total yield of plant, as noted above. These facts indicate that the large kernels are produced to an appreciable extent on those plants whose culms produce high yields

	0– .1	.1– .2	.2– .3	.3– .4	.4– .5	.5– .6	.6– .7	.7– .8	.8– .9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	
3.5– 4.5	2																		2
4.5– 5.5	2																		2
5.5– 6.5																			0
6.5– 7.5	1		1																2
7.5– 8.5	1	1																	2
8.5– 9.5	2																		2
9.5–10.5	4					1													5
10.5–11.5		4	1			2													7
11.5–12.5	7	1	1																9
12.5–13.5	7	7	2																16
13.5–14.5	4	19	13	2															38
14.5–15.5	10	24	26	21	6	4	2												93
15.5–16.5		22	35	38	14	11	4	3			1								128
16.5–17.5	5	10	34	33	32	20	16	5	2		4			1					162
17.5–18.5	1	7	25	27	24	23	14	7	1	3	3	1		1				1	138
18.5–19.5		7	8	16	26	15	13	6	7	1	2			2	1	2		1	107
19.5–20.5	4	2	10	5	8	7	14	2	2	2	1	1	1						59
20.5–21.5			3		4	2	2	2	2	2	3		1						21
21.5–22.5		1		5		1	2			2	1	1							13
22.5–23.5		1		1	1	2	2	2	1			1							11
23.5–24.5			1	1					1		1								4
24.5–25.5		1																	1
25.5–26.5					1														1
26.5–27.5					1														1
27.5–28.5																			0
28.5–29.5																			0
29.5–30.5					1														1
	50	107	160	149	118	88	69	27	16	10	16	4	2	4	1	2	0	2	825

FIG. 44.— *Average weight of kernels per culm in milligrams, subject*
Total yield of culm in grams, relative. 1908
Coefficient of correlation = .464 ± .018

of grain, but not to so great an extent on those plants whose total yield alone is high.

The correlation between average weight of kernels per plant and total number of kernels per plant (Figs. 48, 49, 50), determined for three years, varies from —.253±.032 for 1910 to .071±.034 for 1912; while that between average weight of kernels per plant and average number of kernels per culm per plant (Figs. 51, 52, 53, 54), determined for

	.1–.2	.2–.3	.3–.4	.4–.5	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	
8.5– 9.5	1			1															2
9.5–10.5				1															1
10.5–11.5	1				1														2
11.5–12.5					1		1				1								3
12.5–13.5					1	1				1									3
13.5–14.5		1		3	2	1	6	2	1		1	1							18
14.5–15.5		1	3	2	9	8	11	16	14	13	6	5	3	2					93
15.5–16.5	1		3	5	12	15	17	19	21	21	19	19	12	10	5	2	3	1	185
16.5–17.5		1		3	6	5	17	12	22	14	18	13	13	8	3	2	1		138
17.5–18.5			1	1	1	2	4	3	6	6	5	1	2	5		1			38
18.5–19.5								1	1	3	5			2	2		2		16
19.5–20.5																	1		1
	3	3	7	16	33	32	56	53	65	58	55	39	30	27	10	5	7	1	500

FIG. 45.—*Average weight of kernels per plant in milligrams, subject*
Average yield of culm per plant in grams, relative. 1909
Coefficient of correlation = .337 ± .027

	.3–.4	.4–.5	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	
11.5–12.5						1											1
12.5–13.5	1	1	1			1											4
13.5–14.5	2		4		3	3	1	2	1								16
14.5–15.5		1	2	5	8	3	7	13	4	4	1	2					50
15.5–16.5		3	3	5	8	15	12	9	8	6	2	1					72
16.5–17.5	1	1	4	7	11	15	24	13	6	10	10	1					103
17.5–18.5		1	3	5	7	17	11	11	13	7	4	2	2				83
18.5–19.5				3	2	3	7	4	9	5	3	1					37
19.5–20.5			1	1	1	1	2	3	6	2		1				1	19
20.5–21.5				1		1	1	5	1	1							10
21.5–22.5				1	1	1		1									4
22.5–23.5																	0
23.5–24.5									1								1
	4	7	18	28	41	61	65	61	49	35	20	8	2	0	0	1	400

FIG. 46.— *Average weight of kernels per plant in milligrams, subject*
Average yield of culm per plant in grams, relative. 1910
Coefficient of correlation = .225 ± .032

	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	1.9–2.0	
11.5–12.5				1	1	1	1		1							5
12.5–13.5		1	1	5	10	11	11	9	3	3						54
13.5–14.5		2	3	10	9	8	28	43	24	19	8	4				158
14.5–15.5	1	1	2	4	4	5	18	28	22	17	12	10	3	1		128
15.5–16.5				1			6	4	4	9	7	7	3		1	42
16.5–17.5							1	1				3	2		1	8
17.5–18.5										2		1				3
18.5–19.5								1	1							2
	1	4	6	21	24	25	65	86	55	50	27	25	8	1	2	400

FIG. 47.—*Average weight of kernels per plant in milligrams, subject*
Average yield of culm per plant in grams, relative. 1912
Coefficient of correlation = .429 ± .028

	0– 50	50–100	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	500–550	550–600	600–650	650–700	700–750	750–800	
8.5– 9.5	1			1													2
9.5–10.5			1														1
10.5–11.5	1			1													2
11.5–12.5			1		1					1							3
12.5–13.5				2			1										3
13.5–14.5	1	1	5	3	2	3	2		1								18
14.5–15.5	2	5	17	18	12	11	10	3	8	1	4	1		1			93
15.5–16.5	7	15	18	35	25	19	16	14	16	10	5	1	1	2	1		185
16.5–17.5	5	11	18	18	26	17	18	9	4	5	4		1		1	1	138
17.5–18.5	3	2	6	4	9	8	2	2	1						1		38
18.5–19.5		1	2	5	5			2				1					16
19.5–20.5				1													1
	20	35	68	88	80	58	49	30	30	17	13	3	2	3	3	1	500

FIG. 48.—*Average weight of kernels per plant in milligrams, subject*
Total number of kernels per plant, relative. 1909
Coefficient of correlation = .033 ± .030

	0– 50	50–100	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	
11.5–12.5			1								1
12.5–13.5		1	2			1					4
13.5–14.5	1	1	1	5	1	4	1	1	1		16
14.5–15.5		2	7	9	6	15	7	2	1	1	50
15.5–16.5		2	16	12	20	11	7	3	1		72
16.5–17.5		5	22	24	25	11	14	1	1		103
17.5–18.5	1	7	21	22	20	9	3				83
18.5–19.5		1	12	11	5	6	1			1	37
19.5–20.5		2	6	6	3	1		1			19
20.5–21.5			4	5	1						10
21.5–22.5			3	1							4
22.5–23.5											0
23.5–24.5				1							1
	2	21	95	96	81	58	33	8	4	2	400

FIG. 49.—*Average weight of kernels per plant in milligrams, subject*
Total number of kernels per plant, relative. 1910
Coefficient of correlation = −.253 ± .032

	100–150	150–200	200–250	250–300	300–350	350–400	400–450	450–500	500–550	550–600	600–650	650–700	700–750	750–800	800–850	850–900	900–950	950–1000	1000–1050	1050–1100	1100–1150	1150–1200	1200–1250	1250–1300	
11.5–12.5						1	2	1										1							5
12.5–13.5		2	2	7	7	3	2	3	5	5	7	3	3	2	1	1		1							54
13.5–14.5	1	11	4	3	13	6	15	20	10	15	10	12	8	6	11	4	2	5		1	1				158
14.5–15.5	2	3	6	6	8	7	11	12	13	7	9	11	4	6	5	4	6	4			2		1	1	128
15.5–16.5		1	1		1	5	8	4	6	4	1		4		3	2						1		1	42
16.5–17.5						1	1			1	2	1									1	1			8
17.5–18.5					1	1			1																3
18.5–19.5					1		1																		2
	3	17	13	16	31	24	40	40	35	32	29	27	19	14	20	11	8	11	0	1	4	2	1	2	400

FIG. 50.— *Average weight of kernels per plant in milligrams, subject*
Total number of kernels per plant, relative. 1912
Coefficient of correlation = .071 ± .034

four years, varies from —.172 ± .033 for 1910 to .300 ± .021 for 1908. These correlations fluctuate strongly from year to year, and are never very high, although in some years the coefficients are large enough to be of considerable importance, especially when the coefficient is several times its probable error. From this it must be concluded that the relation of average weight of kernels to average and total number of kernels is responsive to environment, and varies independently much of the time. The kernels may increase in size or the number may increase without a corresponding change taking place in the other character.

	1–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	
3.5– 4.5	2										2
4.5– 5.5	2										2
5.5– 6.5											0
6.5– 7.5	1				1						2
7.5– 8.5	1	1									2
8.5– 9.5	2										2
9.5–10.5	4					1					5
10.5–11.5		4	1		1	1					7
11.5–12.5	7	1	1								9
12.5–13.5	11	5									16
13.5–14.5	11	24	3								38
14.5–15.5	24	36	26	4	3						93
15.5–16.5	9	55	42	16	5			1			128
16.5–17.5	9	50	57	32	7	2	4		1		162
17.5–18.5	8	39	44	30	8	5	2	1		1	138
18.5–19.5	4	24	37	25	8	3		3	2	1	107
19.5–20.5	6	15	15	16	4	2	1				59
20.5–21.5	1	4	5	4	4	3					21
21.5–22.5	1	5	3		3	1					13
22.5–23.5	1	1	4	4	1						11
23.5–24.5	1	1		1	1						4
24.5–25.5	1										1
25.5–26.5		1									1
26.5–27.5		1									1
27.5–28.5											0
28.5–29.5											0
29.5–30.5		1									1
	106	268	238	132	46	18	7	5	3	2	825

FIG. 51.— *Average weight of kernels per culm in milligrams, subject*
Total number of kernels per culm, relative. 1908
Coefficient of correlation = .300 ± .021

The correlation between average weight of kernels per plant and average number of spikelets per culm per plant (Figs. 55, 56, 57), determined for

	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	
8.5–9.5		1		1								2
9.5–10.5				1								1
10.5–11.5	1			1								2
11.5–12.5				1	1				1			3
12.5–13.5				2			1					3
13.5–14.5	1	1	3	2	6	3		2				18
14.5–15.5	1	5	10	14	22	21	11	7	2			93
15.5–16.5	1	6	18	30	33	31	30	22	8	5	1	185
16.5–17.5	1	3	10	25	31	25	26	11	4	2		138
17.5–18.5	1	2	3	8	9	6	5	3	1			38
18.5–19.5				2	6	2	3	2	1			16
19.5–20.5								1				1
	6	18	44	87	108	88	76	48	17	7	1	500

FIG. 52.—*Average weight of kernels per plant in milligrams, subject Average number of kernels per culm per plant, relative. 1909 Coefficient of correlation = .121 ± .030*

	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	
11.5–12.5						1			1
12.5–13.5	1	1	1		1				4
13.5–14.5	2		4	4	3	3			16
14.5–15.5		4	8	8	14	10	4	2	50
15.5–16.5	2	5	14	21	15	11	4		72
16.5–17.5	3	9	21	36	13	15	6		103
17.5–18.5	3	7	26	18	19	7	3		83
18.5–19.5		3	10	11	10	3			37
19.5–20.5	1	2	3	10	1	1		1	19
20.5–21.5		1	6	2	1				10
21.5–22.5	1	1	2						4
22.5–23.5									0
23.5–24.5			1						1
	13	33	96	110	77	51	17	3	400

FIG. 53.—*Average weight of kernels per plant in milligrams, subject Average number of kernels per culm per plant, relative. 1910 Coefficient of correlation = —.172 ± .033*

	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	120–130	
11.5–12.5					1	2	1	1			5
12.5–13.5		1	1	12	7	18	10	4	1		54
13.5–14.5		2	9	13	18	45	46	18	5	2	158
14.5–15.5	1	1	5	7	22	40	27	19	5	1	128
15.5–16.5			1	2	6	10	10	10	2	1	42
16.5–17.5				1		1	3	2	1		8
17.5–18.5					2	1					3
18.5–19.5				2							2
	1	4	16	37	56	117	97	54	14	4	400

FIG. 54.— *Average weight of kernels per plant in milligrams, subject Average number of kernels per culm per plant, relative. 1912 Coefficient of correlation = .087 ± .033*

	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	
3.5– 4.5	2										2
4.5– 5.5	2										2
5.5– 6.5											0
6.5– 7.5	1				1						2
7.5– 8.5		1	1								2
8.5– 9.5		2									2
9.5–10.5	2	2					1				5
10.5–11.5		2	1	2	1	1					7
11.5–12.5	3	4	2								9
12.5–13.5	4	8	3	1							16
13.5–14.5	4	21	13								38
14.5–15.5	5	33	33	15	4	3					93
15.5–16.5	2	32	50	25	16	1	2				128
16.5–17.5	3	26	38	45	37	7	4	2			162
17.5–18.5	2	26	29	36	26	12	3	2	1	1	138
18.5–19.5		15	21	34	15	15	2	3	2		107
19.5–20.5	2	7	12	18	10	9	1				59
20.5–21.5		2	3	3	7	5	1				21
21.5–22.5		1	4	4	3	1					13
22.5–23.5		1	3	4	2	1					11
23.5–24.5		2			1	1					4
24.5–25.5	1										1
25.5–26.5			1								1
26.5–27.5			1								1
27.5–28.5											0
28.5–29.5											0
29.5–30.5		1									1
	33	186	215	187	123	56	14	7	3	1	825

FIG. 55.— *Average weight of kernels per culm in milligrams, subject Average number of spikelets per culm, relative. 1908 Coefficient of correlation = .329 ± .021*

	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	
8.5– 9.5		1		1						2
9.5–10.5				1						1
10.5–11.5		1			1					2
11.5–12.5				1	1			1		3
12.5–13.5			1	1			1			3
13.5–14.5	1	2	3	2	5	4	1			18
14.5–15.5	2	5	9	23	26	20	7	1		93
15.5–16.5	3	8	22	38	34	39	24	14	3	185
16.5–17.5	2	8	16	26	33	26	20	7		138
17.5–18.5		3	6	4	10	9	5	1		38
18.5–19.5				3	5	3	3	2		16
19.5–20.5						1				1
	8	28	57	100	115	102	61	26	3	500

FIG. 56.—*Average weight of kernels per plant in milligrams, subject*
Average number of spikelets per culm per plant, relative. 1909
Coefficient of correlation = .120 ± .030

	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	
11.5–12.5								1	1
12.5–13.5		1	2		1				4
13.5–14.5	2	1	1	2	5	5			16
14.5–15.5	1	2	3	10	15	11	5	3	50
15.5–16.5	1	4	11	19	17	11	8	1	72
16.5–17.5	3	5	13	28	28	17	7	2	103
17.5–18.5	2	1	22	17	21	13	7		83
18.5–19.5		3	3	10	13	7	1		37
19.5–20.5		2	1	5	8	2		1	19
20.5–21.5		1	2	5	2				10
21.5–22.5		1	1	1	1				4
22.5–23.5									0
23.5–24.5					1				1
	9	21	59	97	112	66	28	8	400

FIG. 57.—*Average weight of kernels per plant in milligrams, subject*
Average number of spikelets per culm per plant, relative. 1910
Coefficient of correlation = —.099 ± .033

three years, varies from —.099±.033 for 1910 to .329±.021 for 1908. This is a fluctuating correlation which is not high enough to be of importance, with the exception of that for 1908. This indicates that neither the large nor the small kernels tend strongly to be produced on those plants whose average culm yield is large or small.

The correlation between average weight of kernels per plant and average number of kernels per spikelet per plant (Figs. 58, 59), determined for

	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	1.9–2.0	2.0–2.1	2.1–2.2	2.2–2.3	2.3–2.4	2.4–2.5	2.5–2.6	2.6–2.7	
8.5– 9.5							1		1												2
9.5–10.5											1										1
10.5–11.5	1							1													2
11.5–12.5									1				2								3
12.5–13.5									1			2									3
13.5–14.5								1	3	6	3	1	2	2							18
14.5–15.5							4	4	9	12	16	17	16	12	1				1	1	93
15.5–16.5	1				1	2	3	9	13	24	24	40	38	18	8	3			1		185
16.5–17.5					1		4	7	2	19	24	31	29	10	5	2	2	1		1	138
17.5–18.5					1	1	1	1	1	7	8	12	2	2	1		1				38
18.5–19.5									1	6	3	2	2		2						16
19.5–20.5																		1			1
	2	0	0	0	3	3	13	23	32	74	79	105	91	44	17	5	3	2	2	2	500

FIG. 58.— *Average weight of kernels per plant in milligrams, subject*
Average number of kernels per spikelet per plant, relative. 1909
Coefficient of correlation = .120 ± .030

	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	1.9–2.0	
11.5–12.5					1							1
12.5–13.5			1		1			1	1			4
13.5–14.5				1	6	2	1	2	3	1		16
14.5–15.5			1	1	2	6	10	13	11	3	3	50
15.5–16.5			1	2	4	12	14	26	9	3	1	72
16.5–17.5	1		1	4	11	15	22	24	22	2	1	103
17.5–18.5		1	2	5	12	12	21	18	8	3	1	83
18.5–19.5				1	7	5	14	3	4	3		37
19.5–20.5		1			3	4	5	6				19
20.5–21.5					2	2	5	1				10
21.5–22.5			2	2								4
22.5–23.5												0
23.5–24.5				1								1
	1	2	8	17	49	58	92	94	58	15	6	400

FIG. 59.—*Average weight of kernels per plant in milligrams, subject*
Average number of kernels per spikelet per plant, relative. *1910*
Coefficient of correlation = —.190 ± .033

only two years, varies from —.190±.033 for 1910 to .120±.030 for 1909. These coefficients are not large enough to be of importance in either case, and indicate that the large and the small kernels appear about equally in spikelets containing many or few kernels.

The correlation between average weight of kernels per plant and number of culms per plant (Figs. 60, 61, 62), determined for three years, varies

	1	2	3	4	5	6	7	8	9	10	11	
8.5– 9.5		1		1								2
9.5–10.5		1										1
10.5–11.5	1			1								2
11.5–12.5			1	1	1							3
12.5–13.5				3								3
13.5–14.5		3	4	7	3		1					18
14.5–15.5		9	23	31	18	4	5	3				93
15.5–16.5	5	14	39	80	22	14	7	4				185
16.5–17.5	5	14	32	54	17	11	3		1		1	138
17.5–18.5	1	6	8	16	3	1	2		1			38
18.5–19.5	1	1	8	4		1	1					16
19.5–20.5		1										1
	13	50	115	198	64	31	19	7	2	0	1	500

FIG. 60.—*Average weight of kernels per plant in milligrams, subject*
Number of culms per plant, relative. *1909*
Coefficient of correlation = —.021 ± .030

	2	3	4	5	6	7	
11.5–12.5	1						1
12.5–13.5		2	2				4
13.5–14.5	2	2	9	2	1		16
14.5–15.5	4	12	25	9			50
15.5–16.5	7	22	29	11	3		72
16.5–17.5	12	33	47	6	4	1	103
17.5–18.5	14	34	28	6	1		83
18.5–19.5	5	14	16	1	1		37
19.5–20.5	5	9	3	2			19
20.5–21.5		5	5				10
21.5–22.5		1	2	1			4
22.5–23.5							0
23.5–24.5			1				1
	50	134	167	38	10	1	400

FIG. 61.— *Average weight of kernels per plant in milligrams, subject Number of culms per plant, relative. 1910 Coefficient of correlation = .184 ± .033*

	2	3	4	5	6	7	8	9	10	11	12	13	14	
11.5–12.5				4				1						5
12.5–13.5		3	16	4	7	13	10	1						54
13.5–14.5	2	11	20	35	23	24	30	7	4	1			1	158
14.5–15.5	2	9	17	19	21	17	25	11	4	1	2			128
15.5–16.5		2	6	13	6	6	5	2		2				42
16.5–17.5			1	1	2	1		1	1	1				8
17.5–18.5			1	1	1									3
18.5–19.5				1	1									2
	4	25	61	78	61	61	70	23	9	5	2	0	1	400

FIG. 62.— *Average weight of kernels per plant in milligrams, subject Number of culms per plant, relative. 1912 Coefficient of correlation = .050 ± .034*

from —.021±.030 for 1909 to .184±.033 for 1910. This again is fluctuating and weak, which signifies that both large and small kernels are found on plants that produce few or many culms.

Considering the relation of average weight of kernels per plant to other plant characters as a whole, it is evident that kernel weight is not correlated closely and consistently with any other character here considered. The highest and most stable correlation found is that with average yield of culm per plant, in which the correlation is always positive and rather

	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	1.9–2.0	2.0–2.1	2.1–2.2	2.2–2.3	2.3–2.4	2.4–2.5	2.5–2.6	2.6–2.7	
10–15	1							2		1	2					1				1	8
15–20	1				2		2	6	2	5	1	4	1	2			1	1			28
20–25						1	4	2	8	15	9	7	6	2			2		1		57
25–30					1	1	4	5	12	16	17	21	12	6	4	1					100
30–35						1		4	5	23	23	22	26	8	2					1	115
35–40							3	2	4	11	17	23	23	15	2	1		1			102
40–45								2	1	2	6	23	14	6	6				1		61
45–50										1	4	5	9	3	2	2					26
50–55														2	1						3
	2	0	0	0	3	3	13	23	32	74	79	105	91	44	17	5	3	2	2	2	500

FIG. 63.—*Average number of spikelets per culm per plant, subject*
Average number of kernels per spikelet per plant, relative. 1909
Coefficient of correlation = .324 ± .027

high. In all other cases, however, the correlation varies in the different years from a negative correlation, sometimes of appreciable value, to a comparatively high positive correlation. It is evident, therefore (1) that average weight of kernels in oats has no necessary dependent relation to yield, total and average number of kernels, average number of spikelets and of kernels per spikelet, and number of culms, and (2) that the relation which does exist is largely responsive to environment. It seems that when development is arrested by environmental conditions, yield is reduced by reduction in number of kernels per plant, per culm, and per spikelet, rather than in average weight of kernels and in number of spikelets produced.

Correlation of average number of spikelets per culm per plant with other characters

The correlation between average number of spikelets per culm per plant and average number of kernels per spikelet per plant (Figs. 63, 64), determined for two years, varies from .253±.032 for 1910 to .324±.027 for 1909. This is rather high and fairly constant. From this it may be concluded that there is considerable relation between the number of spikelets produced and the number of kernels produced in these spikelets, an increase in one being associated with an increase in the other.

	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	1.9–2.0	
15–20		1		1	3	2		1	1			9
20–25			2	1	6	5	6		1			21
25–30			4	1	8	10	16	11	7	1	1	59
30–35		1	1	6	12	19	25	14	14	2	3	97
35–40			1	4	9	15	30	33	12	6	2	112
40–45	1			2	10	4	10	20	16	3		66
45–50				2		2	5	11	6	2		28
50–55					1	1		4	1	1		8
	1	2	8	17	49	58	92	94	58	15	6	400

Fig. 64.— *Average number of spikelets per culm per plant, subject*
Average number of kernels per spikelet per plant, relative. 1910
Coefficient of correlation = .253 ± .032

The correlation between average number of spikelets per culm per plant and number of kernels per culm (Fig. 65) was determined for only one

	1– 10	10– 20	20– 30	30– 40	40– 50	50– 60	60– 70	70– 80	80– 90	90–100	
1– 5	33										33
5–10	63	123									186
10–15	7	120	87	1							215
15–20	2	17	132	36							187
20–25	1	7	18	78	14	5					123
25–30		1	1	17	29	6	1	1			56
30–35					3	6	4	1			14
35–40						1	2	1	3		7
40–45								2		1	3
45–50										1	1
	106	268	238	132	46	18	7	5	3	2	825

FIG. 65.— *Number of spikelets per culm, subject*
Total number of kernels per culm, relative. 1908
Coefficient of correlation = .880 ± .005

year, 1908, when it was .880±.005. This indicates a very high correlation between these two characters, from which it may be concluded that the spikelet and kernel development of the plant are mutually related.

Considering as a whole the relation of the average number of spikelets per culm per plant with other characters, it has been seen that as the spikelets increase or decrease there is likewise a strong, simultaneous increase or decrease in the number of kernels produced, and a fairly strong similar change in number of kernels per spikelet.

Correlation of number of culms per plant with other characters

The correlation between number of culms per plant and average yield of culm per plant (Figs. 66, 67, 68), determined for three years, varies

	.1–.2	.2–.3	.3–.4	.4–.5	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	
1	1			1	1	3	3	3									1		13
2	2	3	3	5	8	3	7	5	5	4	2		1	1			1		50
3				5	14	11	16	20	12	14	12	5		4	2				115
4			3	5	10	12	22	17	29	27	22	18	11	10	5	3	3	1	198
5			1			3	5	4	12	6	7	7	10	5	3	1			64
6							3		2		8	8	5	3		1	1		31
7								2	4	6	2	1	1	2			1		19
8								1	1	1	1		2	1					7
9								1						1					2
10																			0
11											1								1
	3	3	7	16	33	32	56	53	65	58	55	39	30	27	10	5	7	1	500

FIG. 66.— *Number of culms per plant, subject*
Average yield of culm per plant in grams, relative. 1909
Coefficent of correlation = .387. ± .026

	.3–.4	.4–.5	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	
2	1	2	5	1	5	6	11	6	5	4	2	1	1				50
3		3	8	11	20	25	18	16	18	7	4	3	1				134
4	2	1	5	10	11	24	29	31	17	21	13	2				1	167
5		1		4	3	6	5	7	7	3	1	1					38
6				2	2		2	1	2			1					10
7	1																1
	4	7	18	28	41	61	65	61	49	35	20	8	2	0	0	1	400

Fig. 67.—*Number of culms per plant, subject*
Average yield of culm per plant in grams, relative. 1910
Coefficient of correlation = .070 ± .034

	.5–.6	.6–.7	.7–.8	.8–.9	.9–1.0	1.0–1.1	1.1–1.2	1.2–1.3	1.3–1.4	1.4–1.5	1.5–1.6	1.6–1.7	1.7–1.8	1.8–1.9	1.9–2.0	
2				2				2								4
3			4	9	1	3	3	1	2	1		1				25
4	1	3	1	4	7	8	11	10	2	5	5	3	1			61
5		1	1	2	6	1	13	20	13	12	5	3	1			78
6				1	5	4	12	13	11	7	4	2	1	1		61
7				1	3	6	14	13	12	5	2	4			1	61
8				2	2	3	9	20	9	12	5	7	1			70
9							1	5	3	7	5	2				23
10							1		3	1	1	1	1		1	9
11								1				1	3			5
12								1				1				2
13																0
14							1									1
	1	4	6	21	24	25	65	86	55	50	27	25	8	1	2	400

Fig. 68.—*Number of culms per plant, subject*
Average yield of culm per plant in grams, relative. 1912
Coefficient of correlation = .383 ± .029

from .070 ± .034 for 1910 to .387 ± .026 for 1909. This is a fluctuating correlation which is only fairly high in any case. From this it may be concluded that the relation between culm production in the plant and the yield of the separate culms is not very close, and is also subject to modification by varying environmental conditions. It was seen above, however, that the relation of number of culms to total yield of plant is very high, from which it is clear that the separate culms of a plant vary considerably among themselves, thereby reducing the correlation when the average culm yield is considered.

The correlation between number of culms per plant and average number of kernels per culm per plant (Figs. 69, 70, 71), determined for three years, varies from .132±.033 for 1910 to .423±.025 for 1909. This again is a fluctuating correlation, not particularly high in any case. It indicates that the relation between the number of culms produced and the number

	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	
1	1	1	2	7	1				1			13
2	5	7	9	12	6	8	2	1				50
3		4	18	24	35	19	11	4				115
4		5	15	36	43	38	25	23	9	3	1	198
5		1		4	15	14	13	10	6	1		64
6				2	4	1	15	7		2		31
7				1	3	7	6		1	1		19
8					1	1	2	3				7
9				1			1					2
10												0
11							1					1
	6	18	44	87	108	88	76	48	17	7	1	500

FIG. 69.—*Number of culms per plant, subject*
Average number of kernels per culm per plant, relative. 1909
Coefficient of correlation = .423 ± .025

	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100	
2	5	3	10	16	9	4	3		50
3	2	17	42	38	25	8	1	1	134
4	4	10	33	43	35	30	11	1	167
5	1	2	8	11	7	6	2	1	38
6		1	3	2	1	3			10
7	1								1
	13	33	96	110	77	51	17	3	400

FIG. 70.—*Number of culms per plant, subject*
Average number of kernels per culm per plant, relative. 1910
Coefficient of correlation = .132 ± .033

	30–40	40–50	50–60	60–70	70–80	80–90	90–100	100–110	110–120	120–130	
2			1	1	1	1					4
3			11	4	4	2	2	2			25
4	1	3	1	12	12	16	9	6	1		61
5		1	2	6	10	26	23	10			78
6			1	6	12	16	19	5	1	1	61
7				3	9	26	15	5	2	1	61
8				4	7	21	18	13	5	2	70
9				1		5	8	9			23
10						2	3	1	3		9
11						1		2	2		5
12						1		1			2
13											0
14					1						1
	1	4	16	37	56	117	97	54	14	4	400

FIG. 71.—*Number of culms per plant, subject*
Average number of kernels per culm per plant, relative. 1912
Coefficient of correlation = .408 ± .028

of kernels per culm may on occasion be high, but is modified by environmental conditions.

Considered as a whole, the relation of number of culms per plant to the other plant characters dealt with has been shown to be fluctuating, generally to a considerable degree, being influenced by different environmental conditions. The correlation is not especially high in any case, except when total plant yield is considered. The conclusions may be drawn, then, (1) that when conditions are favorable, the number of culms will vary to a large extent directly with height of plant, average culm yield, and average number of kernels, but that this relation may, on occasion, entirely disappear; and (2) that the number of culms will vary directly and constantly with the total plant yield; but (3) that the number of culms and the average weight of kernels move practically independently of each other under all conditions.

Constants for 1910 compared with other years

The correlation coefficients for the year 1910 are worthy of special consideration. It is seen that with one exception the only negative coefficients found occur in this one year, five appearing in 1910. The coefficients for 1910 are also noticeably lower, as a general rule, than those of the other years. Of the twenty-two cases involved, there are sixteen that are lower than those of the correlation coefficients determined between the same characters in different years, while only one is higher than all others, and five are intermediate. This condition in 1910 seems to be due to the influence of undetermined environmental conditions on the development of the plants and their seeds. As stated earlier in this paper, this crop was grown on a different, less fertile, soil than were the crops for the other years. This, together with the climatic conditions of 1910, no doubt had the most to do with affecting the correlation coefficients.

By comparing the coefficients of correlation, however, for the years 1908 and 1910, it is seen that those for 1908 are never much lower, but frequently are considerably higher, than those for 1910. The coefficients for 1908 are generally high in comparison with those of all the other years. Those for 1910, on the other hand, were low in comparison, as stated above. It seems, therefore, that in both 1908 and 1910 there were conditions operating which prevented maximum vegetative development and reduced the yield, in the manner stated above; but that these conditions had a different effect on the correlations, increasing, or failing to reduce markedly, those for 1908, and generally decreasing those for 1910. Such results would be expected if in 1908 the plants were grown on poor soil or were crowded but were subject to good weather conditions, and if in 1910 the plants were grown on more fertile soil or in less crowded conditions but at a critical time in the development of the kernels adverse weather conditions prevailed. The former premise would be in agreement with the results of Myers (1912), who found, when working with wheat, that correlations are higher in plants grown on poorer soil, weather conditions being the same. It has been shown above that the culms used in 1908 were from plants grown in drill rows, thus agreeing with the supposed conditions. In 1910 also, as shown above, the plants were given more room than in 1908, but the same room as in other years, while the weather conditions were not favorable.

DISCUSSION OF RESULTS

The means indicate that the growing conditions were not so favorable for the 1910 crop as for those of 1909 and 1912, and resulted in smaller plants and a reduced total yield of grain; but that these smaller plants produced a larger number of spikelets (compared with 1909 only) and larger kernels. The plants of the 1908 crop were grown in drill rows, in more crowded conditions than were those of other years, with the result that the culms were not so tall and the number of spikelets, number of kernels, and yield were all less, but the kernels were practically as large as, or larger than, in any other year except 1910.

It seems that when development is arrested by environmental conditions, yield is reduced by a reduction in number of kernels per plant, per culm, and per spikelet, rather than in average weight of kernels and in number of spikelets produced.

Coupled with the less plant development and yield of 1908 and 1910, there is less variability, as indicated by the standard deviation, in number of culms, total and average number of kernels, total yield, average number of spikelets, and average number of kernels per spikelet. In average weight of kernels, however, there is greater variability in 1908 and 1910.

The relation of these correlation factors to oat production

The value of correlation in breeding and development work has been pointed out from time to time. When certain characters are associated to a high degree, it is possible to so select plants as to increase one character and at the same time increase another because of this correlation.

The results of these studies are of importance in the breeding and production of oats. In the beginning of this paper the question was raised whether the tallest plants produce the most grain. Data have been presented to show that there is a high correlation between these characters so far as this pure line is concerned. This indicates that the growth factors influence the developing plants in such a way that tall plants and high yield of grain are found together.

If these correlations represent the truth, on the whole, for oats during the years represented, and if it were desired to save seed from a field, it would be more advantageous to save seed from the tallest plants; for in so doing one would naturally be selecting seed from the heaviest producers as well.

It is also interesting and important to note that as the plants tend to increase in height, the number of culms also increases. At the same time it is shown also that the average yield per culm increases as the height of plant increases. This shows the possibility of obtaining plants with many culms that may be high in production, which is rather important, since it is sometimes thought that the average yield of culm would tend to decrease as the number of culms on the plant increased.

Another important fact brought out by this study is the relation between average weight of kernels and height of plant, and average weight of kernels and total yield. This has an important bearing on the practice of seeding oats.

The question of size of seed is one that has been much discussed. The common advice given to a grower is that he should reclean his seed in order to obtain only the largest and plumpest for sowing. Many experiments have been conducted in order to determine the relative value of light and heavy seed. Among these, and possibly the best known of all such experiments, are those under the direction of Professor C. A. Zavitz, at Guelph. In these experiments Professor Zavitz worked with hand-picked seed, thus being sure to have a good separation of light and heavy grains. The results show a good gain in yield per acre from the heavy seed. Other results with small grains have been obtained at the Ohio Agricultural Experiment Station (Williams and Welton, 1911 and 1913), and also at the Nebraska Agricultural Experiment Station (Montgomery, 1908). These results do not agree with those found by Professor Zavitz, since no marked results were obtained by sowing larger seed. The difference between the seed used by Professor Zavitz and that sown at the Ohio and Nebraska Stations was that the former was hand-picked and the latter was separated by the use of a fanning mill.

One of the writers has sown hand-picked seed of a number of varieties of oats, and found that in every case the larger yield was obtained from the heavy seed. In another test with oats, in which large and small kernels from the same head were compared, the large seed gave a greater yield.

Although there have been many experiments comparing large and small seed, few studies have been made to determine the parentage of the large seed. Do large seeds come from large plants, or do the smaller, low-yielding plants produce a large percentage of heavy seed? Another point along this line that needs consideration is the relative value of small seed from large plants, as compared with large seed from small plants.

Waldron has made a valuable contribution to the study of light and heavy seed. His data were taken on oats, but he also made some calculations on data taken by Dr. T. L. Lyon and reported in Bulletin 78 of the United States Bureau of Plant Industry. Waldron's paper shows the value of the statistical method of analysis in dealing with such problems. He suggests the importance of a closer analysis of our plants in order to determine some of the fundamental truths that may serve as a basis for improvement. The results, especially with respect to oats, reported in this paper, cast some doubt on the wisdom of planting only the heaviest seed. The data as reported by Waldron show that the heaviest seed come from the smaller rather than from the larger plants. This immediately raises the question as to whether one can reasonably expect gains from the largest seed.

Waldron arranged his data in correlation tables, and determined the correlation between the following characters: average weight of seed and number of grains; average weight of seed and length of head; average weight of seed and length of culm. The calculations showed a negative correlation of —0.595±0.013 between average weight of seed and number of grains; while for average weight of seed and length of head, and average weight of seed and length of culm, correlation coefficients of —0.511±0.015 and —0.404±0.017, respectively, were found. The constants show that the larger kernels are borne by short plants having short heads and producing only a small number of kernels per head; or, in other words, the smallest plants are the ones that produce the heaviest seed. From these data, then, it seems possible that in sowing the heaviest seed one is not using seed from the best-yielding plants.

Some results presented in this paper are of interest in this connection. These studies have to do with the relation between tall plants and average weight of seed; and heavy-yielding plants and average weight of seed. If there is a tendency for certain plants to produce large seed and at the same time be taller or higher-yielding than the average plants of a population, then when heavy seed is selected one would at the same time be selecting tall, high-yielding plants. If, on the other hand, there is no correlation between average weight of seed and height or yield, so that the large seeds are borne in equal amounts by tall and short plants and by heavy- and light-yielding plants, then when heavy seed is selected one is at the same time selecting mediocre plants or those tending to represent the average of the race. Under this condition one would not expect much increase in yield from large seed, while, if the other condition mentioned above holds, it would be natural to expect increase in yield from large seed.

When one compares the correlation coefficients for average height of plant and average weight of kernels for the years 1909, 1910, and 1912[5] — which are .219 ± .029, —.023 ± .034, and .217 ± .032, respectively — some information is obtained concerning the question of light and heavy seed. For the two years 1909 and 1912 the coefficients are practically the same, while for 1910 the coefficient is negative and at the same time it is so low that it shows no relation between these characters for this year. These coefficients show that to some extent the taller plants tend to produce kernels of larger average size for two years, while for the other year this condition does not hold. If these facts represent the average condition for oats for the years 1909 and 1912, were one to select large seed from these crops for planting he would also be selecting plants that are taller. These might have a tendency to produce heavier-yielding plants the next year. On the other hand, if seed were selected from the 1910 crop, tall and short plants would be selected in equal numbers, and the tendency on the whole would be for the plants of the succeeding crop to represent the average conditions rather than to be larger because of the large seed planted.

The correlation coefficients for yield per plant and average weight of kernels for the same three years are .149 ± .029, .035 ± .034, and .220 ± .032, which are very similar to those for average height of plant and average weight of kernels. For the year 1910 there is no relation as measured by the coefficient of correlation between the two characters in question, while there is some relation between these characters for 1909 and 1912, and, provided these facts are true for oats in general for those years, by selecting larger seed one would be obtaining these from the higher-yielding plants. On the other hand, when one selects seed from the 1910 crop, plants having a tendency to yield high or low may be obtained in equal amounts.

Another correlation which is of importance in a practical way is that between average height of plant and average number of spikelets per culm per plant. This correlation for the two years is very high, which shows that it is entirely possible to have tall plants which at the same time will have a high average number of spikelets per culm. This is important, since there is a good correlation between average number of spikelets per culm and average number of kernels per spikelet. Taking both these correlations into consideration, it is seen that yield may be increased by selecting the taller plants, which will at the same time have a high average number of spikelets per plant associated with a high average number of kernels per spikelet.

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[5] 1908 is omitted, since this represents average weight of kernels per culm rather than per plant.

These correlations indicate that it is possible to use certain vegetative characters as a basis for selection when improvement work is to be considered. If from a mixed population tall plants, with a high average number of spikelets per head and with these spikelets well filled with kernels, are selected, one will be selecting plants that will on the whole be high in production.

More evidence is also given in regard to the question of light and heavy seed. The results seem to indicate that for certain years it may be reasonable to expect heavy seed to give a large yield, while for other years the reverse may be the case.

SUMMARY

From the data herein presented the following conclusions may be drawn:

1. Environmental conditions such as exist in different years cause changes in the means. Conditions that generally result in reduction of plant yield also result in reduction of height, number of kernels, and number of culms, but in increase in size of kernels.

2. Yield is reduced by decrease in number of kernels produced, rather than by decrease in their size.

3. Variability decreases with decrease in the means.

4. Correlations are more or less responsive to environmental conditions, and may be divided into *fluctuating* and *stable,* according to their behavior under differing environments.

5. There are high, positive, and fairly stable correlations between average height of plant and (a) total and average yield, (b) total and average number of kernels produced, (c) average number of spikelets per culm; the correlations between average height of plant and (d) average weight of kernels, (e) number of culms, are fluctuating, being high or low on occasion.

6. There are high, positive, and stable correlations between total yield and (a) culm yield, (b) total and average kernel production, (c) spikelet production, (d) culm production.

7. The average kernel weight is not correlated closely and consistently with any other character here considered, except average culm yield, with which the correlation is fairly high and fairly consistent.

8. The average number of spikelets per culm per plant is correlated (a) fairly highly with the average number of kernels per spikelet; (b) apparently very highly with number of kernels per culm; (c) very highly and stably with average height of plant and total yield; and (d) in a fluctuating manner with kernel weight.

9. The correlations between number of culms per plant and (a) height, (b) culm yield, (c) number of kernels, are fluctuating, varying greatly from high to low; between number of culms per plant and (d) total yield they are high, positive, and stable; between number of culms per plant and (e) average kernel weight they are fairly stable and always low.

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AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

VARIATION AND CORRELATION OF OATS (AVENA SATIVA)

PART II. EFFECT OF DIFFERENCES IN ENVIRONMENT, VARIETIES, AND METHODS ON BIOMETRICAL CONSTANTS

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VARIATION AND CORRELATION OF OATS (AVENA SATIVA)

PART II. EFFECT OF DIFFERENCES IN ENVIRONMENT, VARIETIES, AND METHODS ON BIOMETRICAL CONSTANTS

VARIATION AND CORRELATION OF OATS (AVENA SATIVA)

PART II. EFFECT OF DIFFERENCES IN ENVIRONMENT, VARIETIES, AND METHODS ON BIOMETRICAL CONSTANTS[1]

Clyde E. Leighty[2]

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INTRODUCTION

Biometrical methods have recently come into considerable use in studies of genetics. An increasing number of papers discussing the subject are appearing each year, and work of this kind promises to be an important addition to the literature bearing on variation, correlation, and inheritance. The use of the exact methods of measurement required, and the possession of formulæ by means of which a mass of data may be concisely and accurately expressed, mark distinct advances in the science of genetics. It is difficult, for example, for the mind to grasp the data concerned in the relation of height and yield for a thousand plants, but if such data are expressed as a correlation coefficient in a single number the difficulty disappears.

With increasing use of these biometrical methods, a determination of their exact status in studies of variation, correlation, inheritance, and the like, is of importance. It is important to know the conditions that must exist to make comparable the results obtained, to know the effect of environment and hereditary differences on the constants determined. It is also of importance to know, and to have expressed in a statistical way, the variation and correlation that exist in and between the various important characters of many plants. This is especially true of plants of economic importance on which breeding work is being done.

The studies herein reported are on oats (*Avena sativa*), with which considerable breeding work is being done and which stand in need of a large amount of such work. Biometrical methods are used in this study, the various constants determined being the mean, the standard deviation, the coefficient of variability, the coefficient of correlation, and the probable error of each of these.

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It is important to know these values for any plant for whose improvement breeding efforts are being made. Such statistical data should largely direct the efforts and add definiteness to the operations. The mean enables one to know what any strain or variety is likely to do, on the average, in its various characters. The standard deviation and the coefficient of variability are indices of variation: the former is perhaps the best index of variation that is in use for the same or like characters of different lots of material measured in the same units; the latter is of value when the variations of unlike characters of different lots of material measured in dissimilar units are compared.

Rietz and Smith (1910 : 296)[3] define the correlation coefficient as follows: "The correlation coefficient may be defined as the mean product of deviations of corresponding variates from their mean values in units of the standard deviations." The correlation coefficient is of especial value in relation to selection. In the processes involved in selection for any one character, several other characters may at the same time be modified incidentally. By the modification of such characters the correlation in succeeding generations may be modified in inexplicable ways unless the correlations of the parents are known. Again, the actual work of selecting desirable plants is dependent on the correlations that exist in the plants themselves. For example, if the purpose of selection is to increase yield, the relation of this character to height is often of importance.

These studies are not exhaustive, nor could they well be made so. Certain correlations have been determined for several varieties of oats. No inheritance studies are here included. These results must therefore be considered as being preliminary to such studies, for it is of fundamental importance to know the correlations that exist between the different characters of the parental plants before definite and intelligent progress can be made in the study of inheritance, which is the correlation that exists between parents and offspring.

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[3] Dates in parenthesis refer to "Literature cited," page 215.

In addition to the study of the correlations as they exist in oats, other features have been introduced in order to determine the status of correlation studies, to find out how correlations may be modified, and to determine which correlations are capable of modification. These studies have gone hand in hand with studies of the mean and of variation.

The method used in determining the correlation coefficients herein reported is the short method given by Rietz and Smith (1910). The correlation tables and frequency distributions were made from the data taken on the plants, which had been copied on cards, one card containing all data for the culm or the plant dealt with. The cards for any series were then grouped according to the classes of the subject character, and each group was then divided according to the classes of the relative character. By counting the cards in the final groups and entering the numbers in the proper rectangle, correlation tables were made. All formulæ used are contained in Davenport's "Principles of Breeding" (1907).

CHARACTERS USED

The characters of oats dealt with in these studies are:

Height of culm and average height of plant

The height of culm was measured, in millimeters, from the root crown to the base of the apical spikelet. The average height of plant was determined by dividing the sum of the culm lengths by the number of culms possessed by the plant.

Average length of head per plant

This was obtained by dividing the sum of the head lengths of the plant by the number of culms per plant. The length of head was measured, in millimeters, for each culm, the head being considered as extending from the node marking its origin to the base of the apical spikelet.

Weight of plant

Total weight of plant.— This was obtained usually by weighing the entire plant, exclusive of the root, in centigrams. In some cases it was obtained by adding together the weights of the separate culms of the plant.

Total weight of culm.— This was obtained for a few series by weighing in centigrams, separately, the different culms making up the plant, using the entire culm, exclusive of the root. This is the same as the total weight of plant in cases of single-culmed plants.

Average weight of culm per plant.— This was obtained by dividing the total weight of plant, obtained as described above, by the number of culms per plant. The average weight of culms as herein used is often identical with the total weight of plant, this occurring when a single culm forms the plant.

Number of kernels

This was obtained by counting the number of kernels produced by the entire plant, or in some cases by each culm. The average number of kernels per culm was obtained by dividing the total number per plant by the number of culms of the plant.

Number of spikelets

This was obtained in the same way as was the number of kernels.

Number of kernels per spikelet of plant

This was obtained by dividing the number of kernels per plant by the number of spikelets per plant.

Weight of straw

Total weight of straw for the entire plant.— This was obtained by subtracting the total weight of kernels per plant from the total weight of plant.

Total weight of straw per culm.— This was obtained by subtracting the total weight of kernels per culm from the total weight of culm.

Average weight of straw per culm of plant.— This was obtained by dividing the total weight of straw per plant by the number of culms per plant.

Average weight of kernels per plant

This was obtained by dividing the total weight of kernels per plant by the number of kernels per plant.

Diameter of straw

This was obtained by measuring, in decimillimeters, by means of calipers, the greatest diameter of the second internode below the head of the most important culm of the plant.

Breaking strength of straw

This was determined for the internode measured above. The method employed was to cut a piece of straw to a length of eight centimeters, using the part nearest the root. This piece was then placed across an auger hole in a board, the hole being 5.5 centimeters in diameter. A tiny bucket was then suspended from the middle point of the piece of straw by means of a hook made from wire about one millimeter in diameter. Shot was then poured into the bucket at a uniform rate until the straw broke. A very fine shot, about No. 12, was used. The combined weight of the bucket and the shot required in order to break the straw was considered as the breaking strength of the straw.

Yield

Total yield of plant.— This was determined by weighing, in milligrams, the grain produced by the plant, or by adding together the yields determined for individual culms of the plant.

Total yield of culm.— This was determined in some series by weighing the grain produced by each culm.

Average yield of culm per plant.— This was obtained by dividing the total yield of the plant by the number of culms per plant.

MATHEMATICAL METHODS

In determining the mathematical results herein reported, calculations were made to the fifth decimal place. If the fifth place exceeded five in value the fourth place was increased by one, otherwise the fifth number was dropped. With the exception of the coefficient of variability, the constants are reported with three decimal places, the fourth place, determined as above, being dealt with as was the fifth place above. When the constants reported in this paper were used in the determination of other constants, as the coefficient of variability, they were used as herein reported; that is, with three decimal places.

Rietz and Smith (1910 : 304), following Pearson, make the following statement regarding the significance of the probable errors:

"In the comparison of two statistical results, the difference between the two results compared to its probable error is of great value. In general, we may take the probable error in a difference to be the square root of the sum of the squares of the probable errors of the two results. If the difference does not exceed two or three times the probable error thus obtained, the difference may reasonably be attributed to random sampling. If the difference between the two results is as much as five to ten times the probable error, the probability of such differences in random sampling is so small that we are justified in saying that the difference is significant. In fact a difference of ten times its probable error is certainly significant in so far as there is certainty in human affairs."

This method of determining the error of the difference of statistical results compared has been used in the present paper.

STUDIES TAKEN UP

The following studies have been taken up and are separately considered hereinafter :

1. Comparison of biometrical constants determined for oat plants and for the culms of the same plants.
2. Biometrical comparison of varieties of oats.
3. Comparison of biometrical constants determined for oat plants grown in hills and in drills.
4. Effect of different degrees of crowding on biometrical constants of oats.

The correlation tables for each division are included in the text in proximity to the discussion of the constants determined for each table, except in cases when the same series enters into more than one division.

Comparison of biometrical constants determined for oat plants and for the culms of the same plants

In taking up variation and correlation studies with the cereals, the question arises as to whether the plants should be considered as units or whether the individual culms of the plants may be considered as the units with which to work. If plants are used as units, averages per culm of plant must be dealt with in many instances. If culms are the units, the actual culm measurements are dealt with directly. The feeling may often arise in such work that the measures of variation and correlation may differ somewhat, or even essentially, according to the method used.

Investigators have made use of one or the other method, or both, rather indiscriminately. Waldron (1910) has used culms as units in studies with oats, although practically every culm used by him was the entire plant, due to the conditions under which the oats were grown. Roberts (1911) dealt with culms of wheat as units for the crop of one year, and with plants as units for the crop of the succeeding year. Myers (1911) reported correlations for wheat, plants being used as the units for correlation. The writer (1911) reported data on oats, plants being used as units; but in the same paper some constants worked out by Humbert were quoted, these having been worked out with the culm as the unit of calculation. Atkinson (1912), in some recent and as yet unpublished work, has used the culm as the unit in statistical work with several varieties of wheat.

It seemed important, therefore, in view of the considerable amount of statistical work being done with the cereals, to make some trial of these methods in order to determine their comparative value. Accordingly 300 plants of oats, grown in 1911, three inches apart in rows one foot apart, were chosen for use. These were a pure line (137–6) of the variety Early Champion, which had been grown for several years by the Department of Plant-Breeding at Cornell University. Each culm of each of these 300 plants was measured and recorded separately, the yield and the number of grains produced on each culm being a part of the data taken. There were in all 862 culms produced by the 300 plants, or an average of 2.87+ culms per plant. The numbers of plants, with the different numbers of culms, are as follows:

Number of culms per plant...	1	2	3	4	5	6	7	8
Number of plants............	41	52	144	39	18	4	1	1

The constants for the variation and correlation of certain characters of these plants were then determined, the various culms being dealt with as individuals in the one case and the plants being dealt with as individuals in the other; in the latter case, the average per culm of plant being determined. The means and measures of variation determined by the two methods are given in Table 1. Series 1221 is the series worked with, the individual plants here being the units. Series 22 is the designation given to the culms making up the plants of series 1221, when these separate culms are dealt with as units.

The data in Table 1 will now be considered. The average yield of culm per plant in decigrams has a mean of 7.890 ± .118. The total yield of culm in decigrams has a mean of 8.416 ± .109. The yield of culm is larger by .526 ± .161 decigrams, the difference being only about three times its probable error. Considering standard deviation, it is seen that for the average yield of culm per plant it is 3.034 ± .083, while for the total yield of culm it is 4.728 ± .077. The coefficients of variability are, respectively, 38.45 ± 1.20 and 56.18 ± 1.16. These figures express a considerably greater variability in the yield per culm than in average yield of culm per plant.

TABLE 1. SERIES 1221 AND 22 COMPARED. IN SERIES 1221 THE INDIVIDUAL PLANTS ARE DEALT WITH AS UNITS. IN SERIES 22 THE SEPARATE CULMS OF THE SAME PLANTS ARE DEALT WITH AS UNITS

Series 1221	Series 22
Average yield of culm per plant, in decigrams M 7.890 ± .118 σ 3.034 ± .083 C 38.45 ± 1.20	Yield of culm, in decigrams M 8.416 ± .109 σ 4.728 ± .077 C 56.18 ± 1.16
Average height of plant, in centimeters M 58.683 ± .290 σ 7.462 ± .205 C 12.72 ± .36	Height of culm, in centimeters M 59.971 ± .242 σ 10.519 ± .170 C 17.54 ± .29
Average number of kernels per culm of plant M 55.293 ± .737 σ 18.942 ± .521 C 34.26 ± 1.05	Number of kernels per culm M 58.176 ± .655 σ 28.496 ± .462 C 48.98 ± .97
Average number of spikelets per culm of plant M 31.427 ± .393 σ 10.110 ± .278 C 32.17 ± .97	Number of spikelets per culm M 32.979 ± .338 σ 14.714 ± .238 C 44.62 ± .85
Average weight of straw per culm of plant, in decigrams M 10.190 ± .129 σ 3.317 ± .091 C 32.55 ± .99	Weight of straw per culm, in decigrams M 10.781 ± .109 σ 4.731 ± .077 C 43.88 ± .84
Average weight of kernels per plant, in milligrams M 14.080 ± .066 σ 1.684 ± .046 C 11.96 ± .33	Weight of kernels per culm, in milligrams M 13.851 ± .058 σ 2.540 ± .041 C 18.34 ± .31

The mean for average height of plant in centimeters is less by 1.288 ± .378 than is that for height of culm. The standard deviation in the former case is less by 3.057 ± .266, and the coefficient of variability by 4.82 ± .46. These figures indicate that the culm is slightly higher when culms are considered as units than when plants are considered as units, and that variability is greater by a large amount in the former case.

The mean for average number of kernels per culm of plant is less by 2.883 ± .986 than is that for number of kernels per culm. The standard deviation in the former case is less by 9.554 ± .696, and the coefficient of variability is less by 14.72 ± 1.43. These figures indicate that the average for number of kernels is slightly less, and the variability very much less, when plants are considered as the units of measurement.

The mean for average number of spikelets per culm of plant is less by 1.552 ± .518 than is that for number of spikelets per culm. The standard deviation in the former case is less by 4.604 ± .366, and the coefficient of variability is less by 12.45 ± 1.29. These figures indicate that the average for number of spikelets is very slightly less, and the variability very much less, when plants are used as the units of measurement.

The mean for average weight of straw per culm of plant is less by .591 ± .169 than is that for weight of straw per culm. The standard deviation in the former case is less by 1.414 ± .119, and the coefficient of variability is less by 11.33 ± 1.30. These figures indicate that the average for weight of straw is less by a slight amount, and variability is less by a large amount, when plants are used as the units of measurement.

The mean of average weight of kernels per plant in milligrams is 14.080 ± .066. The mean of weight of kernels per culm is 13.851 ± .058. The weight of kernels per plant in milligrams is greater by .229 ± .088 milligrams, which is less than three times the probable error. The standard deviation for average weight of kernels per plant is 1.684 ± .046, for average weight of kernels per culm 2.540 ± .041. The coefficients of variability are, respectively, 11.96 ± .33 and 18.34 ± .31. These figures denoting variability are greater for the kernels per culm than for the kernels per plant, coinciding in this with the figures for all other characters here considered; but the values of the means are reversed, being here, in the only instance, higher for a character when plants are used as the units of measurement.

The means here determined are remarkably close together in value. With one exception, however — this being for average weight of kernels —

the means for all characters are greater for the individual culms than for the average per culm of plant. It may be concluded, then, that for statistical work with oats practically the same means for the different characters will be obtained whether the plants are used as units — the average per culm being here determined — or the culms of the same plants are used as units; but that the means will generally be slightly greater for the culms as units.

TABLE 2. CORRELATIONS, SERIES 1221 AND 22. PLANTS OR CULMS AS UNITS

Series 1221	Series 22
Average yield of culm per plant, in decigrams — Average height of plant, in centimeters r = .853 ± .011	Total yield of culm, in decigrams — Total height of culm, in centimeters r = .854 ± .006
Average yield of culm per plant, in decigrams — Average number of kernels per culm of plant r = .952 ± .004	Total yield of culm, in decigrams — Total number of kernels per culm r = .957 ± .002
Average yield of culm per plant, in decigrams — Average number of spikelets per culm of plant r = .931 ± .005	Total yield of culm, in decigrams — Total number of spikelets per culm r = .938 ± .003
Average yield of culm per plant, in decigrams — Average weight of straw per culm of plant, in decigrams r = .856 ± .010	Total yield of culm, in decigrams — Total weight of straw per culm, in decigrams r = .872 ± .006
Average yield of culm per plant, in decigrams — Average weight of kernels per plant, in milligrams r = .493 ± .029	Total yield of culm, in decigrams — Average weight of kernels per culm, in milligrams r = .573 ± .015
Average weight of kernels per plant, in milligrams — Average height of plant, in centimeters r = .325 ± .035	Average weight of kernels per culm, in milligrams — Total height of culm, in centimeters r = .431 ± .019
Average weight of kernels per plant, in milligrams — Average number of kernels per culm of plant r = .250 ± .036	Average weight of kernels per culm, in milligrams — Total number of kernels per culm r = .380 ± .020

The measures of variation, however — the standard deviations and the coefficients of variability — both indicate, consistently throughout, a greater amount of variability where the culms are used as units; in other words, there is less variability in the averages of culm per plant than in the culms making up the plants.

Correlations

In order to obtain a comparison of the correlations that exist when the plant is used as the unit and when the separate culms of the same plant are used individually as the units, seven correlation coefficients were determined for each of the series. The correlation tables are shown in Figs. 72 to 85. The characters correlated and the coefficients determined are given in Table 2. A summary of the differences between the coefficients for the same correlated characters in the two lines is given in Table 3:

TABLE 3. SUMMARY OF DIFFERENCES IN CORRELATION COEFFICIENTS FOR THE SAME CHARACTERS IN THE TWO LINES COMPARED

Characters correlated	Difference	$\frac{\text{Difference}}{\text{P. E. difference}}$
Yield and height	.001 ± .012	0.1
Yield and number of kernels	.005 ± .004	1.2
Yield and number of spikelets	.007 ± .006	1.2
Yield and weight of straw	.016 ± .012	1.3
Yield and average weight of kernels	.080 ± .034	2.4
Average weight of kernels and height	.106 ± .040	2.6
Average weight of kernels and number of kernels	.130 ± .041	3.2

Comparing the coefficients of correlation given in Table 2, it is seen that they are almost identical in the cases when yield is correlated with height, number of kernels, number of spikelets, and weight of straw, the differences in these cases being, respectively, .001 ± .012, .005 ± .004, .007 ± .006, .016 ± .012. The difference in every case is thus seen to be either less than, or practically equal to, the probable error. Of these four pairs of correlation coefficients, those that are most nearly identical are those for yield and height. In series 1221 this coefficient is .853 ± .011, and in series 22 it is .854 ± .006 — a difference of only .001 ± .012, which is but one twelfth its probable error. The correlation coefficients among these four pairs that differ most are for yield of culm and weight of straw. These are .856 ± .010 for series 1221 and .872 ± .006 for series 22. The difference here is .016 ± .012, which is but little larger than its probable error.

Somewhat large differences are found when average weight of kernels is considered in its relation to other characters. For yield and average weight of kernels, the coefficients are .493 ± .029 in series 1221 and .573 ± .015 in series 22; the difference here being .080 ± .034, or a little more than twice its probable error. For average weight of kernels and height, the coefficients are .325 ± .035 in series 1221 and .431 ± .019 in series 22; the difference here being .106 ± .040. For average weight of kernels and number of kernels, the coefficients are .250 ± .036 in series 1221 and .380 ± .020 in series 22; the difference here being .130 ± .041, or a little more than three times its probable error.

Series 1221

	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Totals
0– 1	1										1
1– 2	1		1								2
2– 3	1			2							3
3– 4		2	8	6	1						17
4– 5			14	8	3						25
5– 6			8	17	8	1					34
6– 7		1	3	10	26	6					46
7– 8				4	25	13	1				43
8– 9					9	19	2				30
9–10				1	4	19	6				30
10–11					4	14	12	1			31
11–12						7	3	3			13
12–13					1		3	3	1		8
13–14							3	2			5
14–15							1	3			4
15–16							2	1			3
16–17									2		2
17–18										1	1
18–19									1		1
19–20								1			1
Totals	3	3	34	48	81	79	33	14	4	1	300

FIG. 72.—*Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative*

r = .853 ± .011

If all the pairs of coefficients of correlation are compared (Table 3), it is seen that there are but three pairs which differ by a greater amount than .016, or by an amount greater than practical equality with their probable errors. In the three pairs in which the difference is greater, there is no case when it is greater than about three times the probable error, the greatest difference being .130 ± .041. These differences are not great, but in every one of the seven cases the higher coefficient of the pair is in series 22, or when the culms are used as the units of measurement. A certain small significance may therefore be attached to these differences.

Series 22

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	Totals
0– 1	1	7	8	4	4									24
1– 2		1	13	6	2	4	1	3						30
2– 3	1		2	20	12	5	5	1						46
3– 4				6	30	17	10	2						65
4– 5				2	21	26	14	4						67
5– 6				1	6	30	30	5	1					73
6– 7					4	14	19	12	2					51
7– 8					1	10	32	18	6	2	2			71
8– 9						5	15	34	19	1				74
9–10						1	19	34	21	6				81
10–11							3	18	19	14				54
11–12							1	10	23	8	1			43
12–13							2	5	18	11	1			37
13–14								3	13	13	4			33
14–15							1	1	12	6	3	1		24
15–16								3	3	14	6	1		27
16–17									3	7	7	1	2	20
17–18									2	6	2	2		12
18–19										4	4	2		10
19–20										2	2	1		5
20–21										1	3	2		6
21–22										1		1		2
22–23									1		1	2		4
23–24										1	1			2
28–29											1			1
Totals	2	8	23	39	80	112	152	153	143	97	38	13	2	862

FIG. 73.*— *Correlation between total yield of culm, in decigrams, subject; and total height of culm, in centimeters, relative*

r = .854 ± .006

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*In this figure and in others marked with an asterisk, the classes containing no individuals are omitted in the publication but were taken into account in the calculations.

Series 1221

	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	68–72	72–76	76–80	80–84	84–88	88–92	92–96	96–100	100–104	112–116	116–120	132–136	Totals
0–1	1																											1
1–2		2																										2
2–3			1		2																							3
3–4				3	9	4		1																				17
4–5					1	5	13	2	3	1																		25
5–6							9	9	8	6	2																	34
6–7								2	12	14	3	10	4		1													46
7–8								1	1	2	11	16	8	3		1												43
8–9											1	6	9	10	3	1												30
9–10												1	2	13	6	5	3											30
10–11													1	1	2	7	9	8	2		1							31
11–12														1	2		1	6	2	1								13
12–13																	1	1	3	3								8
13–14																1			1	1		2						5
14–15																		1			1	1		1				4
15–16																	1					1	1					3
16–17																							1	1				2
17–18																									1			1
18–19																										1		1
19–20																											1	1
Totals	1	2	1	3	12	9	22	15	24	23	17	33	24	28	14	15	15	16	8	5	2	4	2	2	1	1	1	300

FIG. 74.*—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative*

r = .952 ± .004

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*See footnote to Fig. 73, page 93.

Series 22

	0– 4	4– 8	8– 12	12– 16	16– 20	20– 24	24– 28	28– 32	32– 36	36– 40	40– 44	44– 48	48– 52	52– 56	56– 60	60– 64	64– 68	68– 72	72– 76	76– 80	80– 84	84– 88	88– 92	92– 96	96–100	100–104	104–108	108–112	112–116	116–120	120–124	124–128	128–132	132–136	136–140	140–144	144–148	Totals
0– 1	5	8	8	2	1																																	24
1– 2		2	4	14	5	1	2	2																														30
2– 3				3	15	16	9	3																														46
3– 4					2	17	20	10	11	4			1																									65
4– 5							3	18	20	14	7	1	1	2		1																						67
5– 6								3	16	16	19	10	5	1	3																							73
6– 7										7	6	19	5	4	6	1	2			1																		51
7– 8								1			5	8	26	7	11	6	4	2							1													71
8– 9												1	4	12	18	20	13	4	1		1																	74
9–10											1			2	12	18	16	10	10	4	6	2																81
10–11											1				2	3	11	11	10	8	5	1	2															54
11–12														1	1		4	4	6	11	8	4	1	1	1	1												43
12–13																	1	1	5	5	10	7	3	5														37
13–14																		1	3	2	3	2	5	5	7	1	3	1										33
14–15																			2		1	2	5	6	1	1	2	2	2									24
15–16																			1				2	4	12	3	4	1										27
16–17																					1	1			5	3	2	4	1	1	2							20
17–18																							1			2	5	2	1	1								12
18–19																												3	3	2	2							10
19–20																												1	1	2			1					5
20–21																									1					2	2		1					6
21–22																												1									1	2
22–23																							1										1	2				4
23–24																										1										1		2
28–29																																			1			1
Totals	5	10	12	19	23	34	34	37	47	41	39	39	42	29	53	49	51	33	38	31	35	19	20	21	28	12	16	15	8	8	6	0	3	2	1	1	1	862

FIG. 75.*—*Correlation between total yield of culm, in decigrams, subject; and total number of kernels per culm, relative*

r = .957 ± .002

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*See footnote to Fig. 73, page 93.

Series 1221

	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	42–44	44–46	46–48	48–50	50–52	52–54	54–56	56–58	58–60	60–62	64–66	70–72	Totals
0– 1	1																													1
1– 2	2																													2
2– 3		1			2																									3
3– 4			1	5	9		1	1																						17
4– 5				1	3	9	5	4	2	1																				25
5– 6				2	1	2	6	6	9	7	1																			34
6– 7							1	2	16	7	7	8	3	1	1															46
7– 8							2			7	11	10	10	1	2															43
8– 9											3	4	10	7	4	2														30
9–10											1	1	3	7	7	5	3	3												30
10–11												1		2	3	6	5	9	3	1			1							31
11–12												1	1		1		2	3	3		1	1								13
12–13																		1	3	3	1									8
13–14															1			1		1		1	1							5
14–15																	1				1		1		1					4
15–16															1							1		1						3
16–17																								2						2
17–18																											1			1
18–19																												1		1
19–20																													1	1
Totals	3	1	1	8	15	11	15	13	27	22	23	25	27	18	20	13	11	17	9	5	3	3	3	3	1	0	1	1	1	300

FIG. 76.*—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative*

r = .931 ± .005

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* See footnote to Fig. 73, page 93.

Series 22

	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	42–44	44–46	46–48	48–50	50–52	52–54	54–56	56–58	58–60	60–62	62–64	64–66	66–68	68–70	70–72	72–74	74–76	76–78	78–80	Totals
0–1	1	6	4	6	4	1	2																																		24
1–2			1	9	5	5	5	2	2		1																														30
2–3					3	6	12	12	8	2	2		1																												46
3–4						2	5	14	22	9	6	4	1	1	1																										65
4–5								2	9	18	14	14	4	2		4																									67
5–6								1	2	6	9	18	15	13	3	3	2	1																							73
6–7											3	5	8	8	12	3	6	1	4		1																				51
7–8								1	1			2	7	10	13	12	8	7	5	1	3					1															71
8–9													2	3	8	9	12	16	9	8	4	3																			74
9–10												1		1		3	11	16	15	12	9	2	5	5					1												81
10–11												1				2	1	4	5	8	12	11	4	3	3																54
11–12																2		2	2	5	4	7	7	7	3	1		2		1											43
12–13																	1	1		2	5	3	9	6	3	3	3	1													37
13–14																1				3	2	2	1	4	5	5	4	1	3	1	1										33
14–15																			1	1	1			2	5	4	3	2		2	1	1	1								24
15–16																				1					3	4	6	7	1	2	3										27
16–17																					1		1		2	3	2	2	1	4	1	1		1		1					20
17–18																								1			1	5	3			1	1								12
18–19																													1	2	1	4	1		1						10
19–20																														1	1	2				1					5
20–21																											1				1	3			1						6
21–22																												1												1	2
22–23																							1											1	1			1			4
23–24																												1									1				2
28–29																																			1						1
Totals	1	6	5	15	12	14	24	32	44	35	35	45	38	38	37	39	41	48	41	41	42	28	28	28	24	21	20	22	10	13	9	12	3	2	4	2	1	1	0	1	862

FIG. 77.*—*Correlation between total yield of culm, in decigrams, subject; and total number of spikelets per culm, relative*

r = .938 ± .003

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*See footnote to Fig. 73, page 93.

Series 1221

	4– 5	5– 6	6– 7	7– 8	8– 9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	22–23	24–25	Totals
0– 1	1																			1
1– 2		2																		2
2– 3	1	1		1																3
3– 4		5	7	3	2															17
4– 5	1	2	12	5	1	2	2													25
5– 6			9	16	5	2		2												34
6– 7				19	12	10	3	1			1									46
7– 8				5	10	15	5	2	2	1	2		1							43
8– 9						12	11	2	4	1										30
9–10						3	12	7	3	2	2	1								30
10–11							9	5	6	8	1			1	1					31
11–12								4	3		2	2	1		1					13
12–13									1	3	1	1		1					1	8
13–14										1		2	1					1		5
14–15													2		2					4
15–16											1	1	1							3
16–17														1		1				2
17–18																	1			1
18–19																	1			1
19–20																	1			1
Totals	3	10	28	49	30	44	42	23	19	16	10	7	6	3	4	1	3	1	1	300

FIG. 78.*—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative*

r = .856 ± .010

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* See footnote to Fig. 73, page 93.

	2– 3	3– 4	4– 5	5– 6	6– 7	7– 8	8– 9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–32	32–33	33–34	34–35	Totals
0– 1	3	6	10	4	1																													24
1– 2		5	11	5	1	4	1	2	1																									30
2– 3	1	1	12	18	6	3	2		2		1																							46
3– 4			4	30	16	8	1	1	1	2				2																				65
4– 5			1	11	24	13	10	2	4	1		1																						67
5– 6				1	17	28	12	9	4	1	1																							73
6– 7					3	10	15	11	3	6	1	2																						51
7– 8					1	7	19	18	11	4	1	1	3	1	2	1		2																71
8– 9						1	12	19	18	11	5	5	2			1																		74
9–10							1	20	26	15	9	3	2	2		3																		81
10–11								1	8	17	16	6	5		1																			54
11–12									6	9	9	7	3	3	3		2										1							43
12–13								1	1	5	6	10	5	3	1	2		1		1					1									37
13–14											6	5	9	7	1	3	2																	33
14–15											3	3	5	2	3	1	2	2	1	2														24
15–16											1	2	5	5	6	1	4	1	1						1									27
16–17													1	2	1	6	2	1		2	1	1	1								2			20
17–18													1	3	1		2		2	1			1	1										12
18–19															1		2	3	1	1	1									1				10
19–20																1	1		1	2														5
20–21																	1	1	1	1	1		1											6
21–22																					1								1					2
22–23																		1			2	1												4
23–24																			1		1													2
28–29																																	1	1
Totals	4	12	38	69	69	74	73	84	85	71	59	45	41	30	20	19	18	12	8	10	7	2	3	1	2	0	1	0	1	1	2	0	1	862

FIG. 79.*—*Correlation between total yield of culm, in decigrams, subject; and total weight of straw per culm, in decigrams, relative*

r = .872 ± .006

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*See footnote to Fig. 73, page 93.

Series 1221

	7– 8	8– 9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	Totals
0– 1	1															1
1– 2			1	1												2
2– 3			1		1		1									3
3– 4				1	1	5	6	3	1							17
4– 5				2	4	6	6	5	2							25
5– 6				1	4	5	10	5	6	2	1					34
6– 7				2	4	10	7	14	7	2						46
7– 8				1	2	4	12	19	3	1			1			43
8– 9					2	1	9	12	4	2						30
9–10						2	6	10	10	1	1					30
10–11					2	3	6	14	4	1	1					31
11–12							1	5	4	1	1		1			13
12–13								3	4	1						8
13–14								2	1		1		1			5
14–15								1	2			1				4
15–16									1	1					1	3
16–17									1	1						2
17–18									1							1
18–19									1							1
19–20								1								1
Totals	1	0	2	8	20	36	64	94	52	13	5	1	3	0	1	300

FIG. 80.—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative*

r = .493 ± .029

	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	Totals
0– 1	1	2	4	2	6		2	4	1				2											24
1– 2			2	3	2	2	4	3	5	5	1	2		1										30
2– 3					1	1	7	4	8	7	11	3	2	1		1								46
3– 4				1	1	1	5	5	10	13	6	13	8	1	1									65
4– 5					4			7	4	20	11	10	8	2	1									67
5– 6							1	5	6	15	13	15	11	5	1	1								73
6– 7						1	1	3	8	5	13	9	6	5										51
7– 8						1		3	5	14	8	21	9	7	2								1	71
8– 9							1		5	9	18	25	8	5	2	1								74
9–10								1	7	16	11	20	18	5	1	1				1				81
10–11									2	7	9	17	12	3	3						1			54
11–12								1	1	2	12	11	7	6	1		1	1						43
12–13										3	5	9	13	3	3	1								37
13–14										4	6	11	4	3	2	3								33
14–15										3	3	3	7	5	1		2							24
15–16												5	13	8				1						27
16–17											3	2	6	6	1		2							20
17–18												1	3	7			1							12
18–19												1	4	4	1									10
19–20													2	2	1									5
20–21													1	3	1			1						6
21–22												1					1							2
22–23														2	1						1			4
23–24														1						1				2
28–29																		1						1
Totals	1	2	6	6	14	6	21	36	62	123	130	179	144	85	23	8	7	4	0	2	2	0	1	862

FIG. 81.*— *Correlation between total yield of culm, in decigrams, subject; and average weight of kernels per culm, in milligrams, relative*

r = .573 ± .015

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*See footnote to Fig. 73, page 93.

Series 1221

	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Totals
7– 8	1										1
8– 9											0
9–10	1			1							2
10–11			2	1	2	2	1				8
11–12			4	4	4	7		1			20
12–13			7	9	9	9	2				36
13–14	1	2	10	9	19	17	6				64
14–15		1	4	17	32	23	10	6	1		94
15–16			5	7	9	15	8	5	2	1	52
16–17			2		4	3	2	1	1		13
17–18					1	2	2				5
18–19							1				1
19–20					1	1		1			3
20–21											0
21–22							1				1
Totals	3	3	34	48	81	79	33	14	4	1	300

FIG. 82.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative*

r = .325 ± .035

Series 22

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	Totals
3– 4		1												1
4– 5				1	1									2
5– 6			1	2	1	1		1						6
6– 7			1	1		1	1	2						6
7– 8	1	2	2	2	1		2	4						14
8– 9				2		1	1	1		1				6
9–10		2	4	3	3	2	4	1	2					21
10–11		2	2	3	6	6	12	2	1		2			36
11–12	1		3	6	5	12	12	12	9	2				62
12–13			5	8	18	20	24	20	17	9	2			123
13–14		1	1	4	19	22	19	24	28	11			1	130
14–15			1	3	14	22	40	37	34	21	5	1	1	179
15–16			2	2	8	15	23	27	29	26	10	2		144
16–17			1	1	4	7	9	12	12	17	14	8		85
17–18						2	3	3	6	6	2	1		23
18–19				1		1		4	1	1				8
19–20							1	1	1	2	1	1		7
20–21									2		2			4
22–23								1		1				2
23–24								1	1					2
25–26							1							1
Totals	2	8	23	39	80	112	152	153	143	97	38	13	2	862

FIG. 83.*— *Correlation between average weight of kernels per culm, in milligrams, subject; and total height of culm, in centimeters, relative*

r = .431 ± .019

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* See footnote to Fig. 73, page 93.

Series 1221

	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	68–72	72–76	76–80	80–84	84–88	88–92	92–96	96–100	100–104	112–116	116–120	132–136	Totals
7–8	1																											1
8–9																												0
9–10		1			1																							2
10–11		1						1	2		1		1		1	1												8
11–12					1	1	3	1	1	2	1	2	3	1	1	1			1		1							20
12–13					3	2	3	1	2	5	2	8	2	2	1	2		2	1									36
13–14			1		5	2	5	5	5	6		8	7	6	2	2	4	5		1								64
14–15				2	1	3	3	3	7	8	10	12	5	9	4	6	8	4	2	3		2		1			1	94
15–16				1	1	1	5	2	5	2	2	1	5	7	3	2	1	4	3	1	1	1	1	1	1	1		52
16–17							2	1	2		1	1		2	1		1					1	1					13
17–18							1					1	1		1				1									5
18–19																		1										1
19–20								1						1		1												3
20–21																												0
21–22																	1											1
Totals	1	2	1	3	12	9	22	15	24	23	17	33	24	28	14	15	15	16	8	5	2	4	2	2	1	1	1	300

FIG. 84.*— *Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative*

$r = .250 \pm .036$

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* See footnote to Fig. 73, page 93.

Series 22

	0– 4	4– 8	8– 12	12– 16	16– 20	20– 24	24– 28	28– 32	32– 36	36– 40	40– 44	44– 48	48– 52	52– 56	56– 60	60– 64	64– 68	68– 72	72– 76	76– 80	80– 84	84– 88	88– 92	92– 96	96–100	100–104	104–108	108–112	112–116	116–120	120–124	128–132	132–136	136–140	140–144	144–148	Totals
3– 4		1																																			1
4– 5			2																																		2
5– 6	1		1	1	1		2																														6
6– 7		1		1		1		2					1																								6
7– 8	2		4	1	1			1		1			1	2		1																					14
8– 9					2			1		1										1					1												6
9–10		2	1	3		4	2	1	3	2					1		1				1																21
10–11	1	2	1	1	2	1	3	2	3	1	5	2	1	1	3	1	2	2				1				1											36
11–12		1		5	2	2	5	4	6	3	1	3	3	3	5	1	4	3	1	2	4	2	1		1												62
12–13			2	3	3	6	7	5	8	9	10	7	4		10	5	8	5	8	3	7		2	4			3	2	2								123
13–14		1			6	6	5	4	6	6	7	11	5	5	5	10	8	5	7	5	7	5	2	2	5	2	1	1		1	2						130
14–15			1	2	2	7	7	5	9	7	6	9	15	6	12	17	15	7	7	11	4	5	5	6	3	1	4	2	1	1	1					1	179
15–16	1	1		1	2	6	2	6	8	6	3	3	7	6	9	11	7	6	4	7	8	3	3	5	10	3	3	5	2	3	1	2					144
16–17		1			1	1		3	4	5	3	3	3	3	5	2	4	3	3	1	2	2	5	4	6	4	5	2	3	2	2		2		1		85
17–18					1		1	1			2		2	1	2	1	1	1	3	1	1				1			2		1		1					23
18–19				1				1				1		1			1	1	2																		8
19–20															1				2		1	1	1					1									7
20–21														1					1						1									1			4
21–22																																					0
22–23											1															1											2
23–24											1												1														2
24–25																																					0
25–26								1																													1
Totals	5	10	12	19	23	34	34	37	47	41	39	39	42	29	53	49	51	33	38	31	35	19	20	21	28	12	16	15	8	8	6	3	2	1	1	1	862

FIG. 85.*—*Correlation between average weight of kernels per culm, in milligrams, subject; and total number of kernels per culm, relative*

r = .380 ± .020

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*See footnote to Fig. 73, page 93.

It can be said, from the results here shown, that practically the same correlation coefficients will be obtained when plants are used as the units of calculations as when the separate culms making up these plants are used as the units, but that there is a uniform tendency for the correlation to be greater when culms are used as the units. Greater differences exist in the correlation when average weight of kernels enters as one of the characters, but none of the differences found are great enough to be of special significance. The correlation coefficients obtained by the several investigators, therefore, whether they worked with culms or with plants, are comparable, with slight reservation, as far as any effect of this usage is concerned. The same may be said for the means, the standard deviations, and the coefficients of variability. All these constants, however, are generally slightly higher when the separate culms are the units than when the plants are the units.

Biometrical comparison of varieties of oats

The results obtained by Waldron (1910) in biometrical work with oats differed radically from those obtained by the writer (1911). The correlations obtained by Waldron between average weight of grain and number of grains per head, length of culm, and length of head, were of considerable value, amounting to forty to sixty per cent, and were negative. Those obtained by the writer for the same characters were large and positive. Some explanation of these discordant results was sought. One suggestion was that the use of different varieties had been the cause. In order to test the theory that correlations may be affected by varietal differences, pure lines of four different varieties were grown side by side under similar conditions during the season of 1911. These pure lines had been grown for several years by the Department of Plant-Breeding in the comparative rod-row tests that it was conducting. Fifteen-gram lots of seed were sown in eighteen-foot rows, and the rows were a foot apart. The varieties used were as follows:

Series	Variety	Pure line
1200	Great American	125–20
1219	Early Champion	137–6
1238	Welcome	123–5
1257	Sixty Day	62–II–6–3

These varieties were all sown on the same day, but were harvested as they ripened. The dates of harvesting and the figures showing thickness of stand are:

Variety	Harvested	Thickness of stand
Great American	July 24, 1911	400 plants in 15 feet = 26.7 plants per foot
Early Champion	July 24, 1911	600 plants in 18 feet = 33.3 plants per foot
Welcome	August 4, 1911	325 plants in 14 feet = 23.2 plants per foot
Sixty Day	July 24, 1911	610 plants in 18 feet = 33.9 plants per foot

It is seen that the Early Champion and the Sixty Day plants were somewhat more crowded in the row than were the Great American and the Welcome. By referring to Table 4, where are given the means for average weight of kernels per plant, it is seen that the means for Early Champion and Sixty Day are 10.913 ± .115 and 13.067 ± .093 milligrams, respectively, and for Great American and Welcome they are 22.640 ± .172 and 25.413 ±.155 milligrams, respectively. The latter two are thus seen to have much larger kernels than the former two, which accounts for the fewer plants growing in the rows of these varieties, since fifteen grams of each variety were sown in rows of the same length.

The size of kernel is a varietal characteristic and is not so much affected by crowded growing conditions as is any other character except height, as will be shown in another part of this paper.

Since the growing conditions of these varieties were slightly different, due to the growing of different numbers of plants in the rows, the differences that are observed in the means and the standard deviations are not surely due to varietal differences. They should be the same, however, as would be obtained were the plants taken from fields where seeding had been at a uniform rate of bushels per acre for different varieties, as has been done by some experimenters.

At the above rate of seeding and under the other conditions of growth, few plants of either of the varieties produced more than one culm per plant. The frequency distributions for numbers of culms per plant are as follows:

Variety	Number of culms per plant		
	1	2	3
1200	300	....	....
1219	295	5	....
1238	295	5	....
1257	288	11	1

It is seen here that in 1200 plants, taken without selection from the rows, there were but 1223 culms.

The means (M), standard deviations (σ), and coefficients of variability (C) for six characters of these different varieties are given in Table 4.

Taking up for consideration first the average yield of culm per plant, it is seen that there is considerable difference in the means. The highest is in series 1238, it being 8.217 ± .219 decigrams; the lowest is 3.433 ± .096, in series 1219. For series 1200 the mean is 6.403 ± .149, and for series 1257 it is 3.950 ± .102. The average yield per culm, which is essentially yield per plant, is, then, for the varieties, in descending order of yield, Welcome, Great American, Sixty Day, Early Champion. The standard deviations follow exactly the same order as do the means, which would indicate that the greatest variation exists in series 1238 and the least in series 1219. The coefficients of variability, however, indicate a different condition. For series 1219 this is 72.04 ± 2.82; for series 1238 it is 68.54 ± 2.62; for series 1257 it is 66.53 ± 2.51; and for series 1200 it is 59.75 ± 2.15. The greatest variability is thus indicated by the coefficient of variability where the standard deviation indicates the least. After an inspection of the frequency distributions for the different series, one is inclined to consider the standard deviation as by far the better index of variability in this case.

TABLE 4.—MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY, SERIES 1200, 1219, 1238, 1257.* VARIETIES

Average yield of culm per plant, in decigrams

	1200	1219	1238	1257
M....	6.403 ± .149	3.433 ± .096	8.217 ± .219	3.950 ± .102
σ....	3.826 ± .105	2.473 ± .068	5.632 ± .155	2.628 ± .072
C.....	59.75 ± 2.15	72.04 ± 2.82	68.54 ± 2.62	66.53 ± 2.51

Average height of plant, in centimeters

	1200	1219	1238	1257
M....	56.533 ± .362	55.850 ± .432	62.916 ± .507	49.566 ± .416
σ....	9.304 ± .256	11.114 ± .306	13.041 ± .359	10.703 ± .294
C.....	16.46 ± .46	19.90 ± .57	20.73 ± .59	21.59 ± .62

Average number of kernels per culm of plant

	1200	1219	1238	1257
M....	26.387 ± .510	29.293 ± .695	30.213 ± .755	28.440 ± .663
σ....	13.113 ± .361	17.867 ± .491	19.420 ± .534	17.054 ± .469
C.....	49.70 ± 1.67	60.99 ± 2.21	64.28 ± 2.39	59.96 ± 2.16

Average number of spikelets per culm of plant

	1200	1219	1238	1257
M....	14.120 ± .275	17.160 ± .359	16.587 ± .421	14.000 ± .330
σ....	7.082 ± .195	9.230 ± .254	10.834 ± .298	8.475 ± .233
C.....	50.16 ± 1.69	53.79 ± 1.86	65.32 ± 2.45	60.54 ± 2.19

Average weight of straw per culm of plant, in decigrams

	1200	1219	1238	1257
M....	8.653 ± .180	5.227 ± .107	9.767 ± .204	5.303 ± .116
σ....	4.632 ± .127	2.763 ± .076	5.250 ± .144	2.977 ± .082
C.....	53.53 ± 1.85	52.86 ± 1.81	53.75 ± 1.86	56.14 ± 1.97

Average weight of kernels per plant, in milligrams

	1200	1219	1238	1257
M....	22.640 ± .172	10.913 ± .115	25.413 ± .155	13.067 ± .093
σ....	4.417 ± .121	2.967 ± .082	3.983 ± .110	2.400 ± .066
C.....	19.51 ± .56	27.19 ± .80	15.67 ± .44	18.37 ± .52

*1200, Great American
1219, Early Champion
1238, Welcome
1257, Sixty Day

The means for average height of plant in centimeters vary among themselves less than for the character just considered. The mean for series 1238 is again the highest, being 62.916 ± .507. Series 1200 is again second highest, being 56.533 ± .362. The order of series 1219 and series 1257 is reversed, the former being here 55.850 ± .432, which is considerably larger than 49.566 ± .416, the mean of the latter. The short plant of the Sixty Day variety is without doubt a varietal characteristic. It seems likely also that the means for height of each of these varieties denote varietal differences. The greatest variability is again in series 1238, as is indicated by the standard deviation of 13.041 ± .359. The least variability in height occurs in series 1200, where the standard deviation is 9.304 ± .256. The short-culmed variety, the Sixty Day, has a standard deviation of 10.703 ± .294, and for series 1219 the standard deviation is 11.114 ± .306.

In average number of kernels per culm of plant the means of the different varieties are not far apart in value. The highest, 30.213 ± .755, is again for series 1238. The lowest, 26.387 ± .510, is for series 1200. Series 1219 is second in order, with a mean of 29.293 ± .695, and series 1257 is third with 28.440 ± .663. The standard deviations for the four series follow the same order of value as do the means, the largest being 19.420 ± .534 for series 1238 and the smallest 13.113 ± .361 for series 1200.

It is seen that the large differences do not exist in the means for number of kernels that exist in the means for yield, neither do the means follow the same order in amount. It is evident, therefore, that increase in yield is not due to a larger number of kernels per culm. The average yields per culm are more nearly proportional to the average weight of kernels per plant. By examining the means of the latter character it is seen that series 1238 has much the largest kernel, the mean being 25.413 ± .155. The mean for yield of the same series is also considerably above the means of the other series. The same condition exists throughout. Series 1219 has the smallest mean for yield and also the smallest mean for average weight of kernels, the latter being but 10.913 ± .115. The greatest variability, however, is in series 1200, where the standard deviation is 4.417 ± .121. The least variation is in series 1257, where the standard deviation is 2.400 ± .066.

The means for number of spikelets do not follow the same order of value as do the means of yield and average weight, neither is the order that of the number of kernels, although it is nearer the latter. Series 1219 here has the largest mean, it being 17.160 ± .359. Series 1257 has the smallest, it being 14.000 ± .330. Series 1238 has the greatest amount of variability, however, the standard deviation here being 10.834 ± .298, while that nearest to it is 9.230 ± .254 for series 1219. The smallest standard deviation is 7.082 ± .195, for series 1200.

Since the means of number of spikelets are not proportional to the means of average yield, the same conclusions hold as were made when number of kernels was discussed. And again, since the means for number of spikelets are not directly proportional to those of number of kernels, it follows that the average number of kernels per spikelet must vary for different varieties. These values have not been determined from the original data, but it is evident from the means here determined that the Sixty Day oats have the largest number of kernels per spikelet and that the Early Champion oats have the smallest number. The remaining varieties have numbers of kernels per spikelet somewhere between these two.

The means for average weight of straw per culm of plant in decigrams follow the same order of value as do the means for average yield of culm per plant in decigrams. Series 1238 has the largest mean, 9.767 ± .204, while series 1219 has the smallest, 5.227 ± .107. For series 1200 and 1257 the means are 8.653 ± .180 and 5.303 ± .116, respectively. The standard deviations again follow the same order as do the means, being greatest in series 1238 and least in series 1219.

While there is a definite rise and fall in the means of the average weight of straw per culm of plant with increase and decrease in the means of the average yield of culm per plant for the different varieties here considered, the proportion of grain to straw is not the same for each variety. Considering the mean for average yield of culm per plant as unity in each case, the mean of average weight of straw per culm of plant is in the following proportions for the different varieties:

Great American	1 : 1.35
Early Champion	1 : 1.52
Welcome	1 : 1.19
Sixty Day	1 : 1.34

As the average yield of culm per plant increases, the proportion of straw to grain decreases for the different varieties, with the exception of the Sixty Day variety. In the Welcome variety the proportion of straw to grain is lower than in any of the others. It seems to be a varietal characteristic of the Welcome to produce less straw to the unit amount of grain.

Considerable difference is shown in average yield of culm per plant by the means determined. This may be due to the difference in stand, caused by the large size of kernels in certain varieties. The means of height of plant seem to denote some varietal differences. The larger yields from certain varieties are not due to a larger number of kernels per culm, or to a larger number of spikelets per culm, as the means for these characters are nearly the same for each variety. The yield per culm seems directly proportional to the average weight of kernels per plant. The average number of kernels per spikelet is largest in the Sixty Day variety and smallest in Early Champion. The proportion of straw to grain differs in the different varieties. As the average yield of culm per plant increases, the proportion of straw to grain decreases — not entirely regularly, however, for the different varieties.

The standard deviation is probably the better index of variability, when the same character of different varieties is being compared and when the same unit of measure has been used. Basing conclusions on this constant, then, it is seen that the greatest variability occurs in the Welcome variety for all characters considered, except average weight of kernels. In this character the variability of this variety is exceeded only by that of the Great American. The least variability occurs in Great American for height, number of kernels, and number of spikelets, in Early Champion for yield and for weight of straw, and in Sixty Day for average weight of kernels. Sixty Day is next to the lowest in variability in all characters considered, except average weight of kernels. The order of value of the standard deviations is the same as that of the means, for yield, number of kernels, and weight of straw; that is, the greatest variability in these characters is found where the means are largest, and decrease in variability takes place in the same order as decrease in means. In the number of spikelets the standard deviation is highest when the mean is next highest, and next highest when the mean is highest. The same condition exists for the lowest and the next lowest standard deviations. Exactly the same conditions hold for average weight of kernels as hold for number of spikelets. For height there is more complication, the lowest standard deviation being associated with the second highest mean, the second lowest standard deviation with the lowest mean, and the second highest standard deviation with the second lowest mean.

Correlations

Coming now to the correlations that exist in these several varieties, it is seen in Table 5 that seven correlation coefficients for each variety have been determined. These are grouped in the table in such a way that the coefficients for each variety are given under a single heading showing the characters correlated.

TABLE 5. CORRELATIONS, SERIES 1200, 1219, 1238, 1257.* VARIETIES

Average yield of culm per plant, in decigrams — Average height of plant, in centimeters			
1200	r = .866 ± .010	1238	r = .889 ± .008
1219	r = .859 ± .010	1257	r = .875 ± .009
Average yield of culm per plant, in decigrams — Average number of kernels per culm of plant			
1200	r = .959 ± .003	1238	r = .985 ± .001
1219	r = .934 ± .005	1257	r = .965 ± .003
Average yield of culm per plant, in decigrams — Average number of spikelets per culm of plant			
1200	r = .961 ± .003	1238	r = .985 ± .001
1219	r = .916 ± .006	1257	r = .954 ± .003
Average yield of culm per plant, in decigrams — Average weight of straw per culm of plant, in decigrams			
1200	r = .818 ± .013	1238	r = .944 ± .004
1219	r = .933 ± .005	1257	r = .925 ± .006
Average yield of culm per plant, in decigrams — Average weight of kernels per plant, in milligrams			
1200	r = .686 ± .021	1238	r = .596 ± .025
1219	r = .539 ± .028	1257	r = .563 ± .027
Average weight of kernels per plant, in milligrams — Average height of plant, in centimeters			
1200	r = .654 ± .022	1238	r = .575 ± .026
1219	r = .553 ± .027	1257	r = .577 ± .026
Average weight of kernels per plant, in milligrams — Average number of kernels per culm of plant			
1200	r = .524 ± .028	1238	r = .494 ± .029
1219	r = .340 ± .034	1257	r = .415 ± .032

* 1200, Great American
1219, Early Champion
1238, Welcome
1257, Sixty Day

For average yield of culm per plant correlated with average height of plant (Figs. 86 to 89), the coefficients are seen to be nearly equal for the several varieties. The highest, .889 ± .008 for series 1238, is but .030 ± .013 higher than the lowest, .859 ± .010 for series 1219. The extreme difference is a little more than twice its probable error.

Series 1200

	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Totals
0– 1	2	3		1								6
1– 2		9	11	6	3							29
2– 3		1	7	12	4							24
3– 4			1	12	9	2		1				25
4– 5				3	22	13						38
5– 6				1	10	20	4					35
6– 7					5	17	10					32
7– 8						6	12	3				21
8– 9		1				6	12	5				24
9–10						2	8	7				17
10–11							6	8	1			15
11–12						1	2	4	3			10
12–13								2	6			8
13–14						1		3				4
14–15							1	1		1		3
15–16								1				1
16–17								1	1			2
17–18										1		1
18–19									1			1
19–20										3	1	4
Totals	2	14	19	35	53	68	55	36	12	5	1	300

FIG. 86.— *Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative. Great American*

r = .866 ± .010

Series 1219

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Totals
0– 1	3	10	12	13	3								41
1– 2			1	13	25	17	3						59
2– 3					6	27	18	7	1				59
3– 4					1	6	17	12	1	1			38
4– 5						1	8	19	7				35
5– 6								7	13	4			24
6– 7							1	3	8	7	1		20
7– 8									5	4			9
8– 9									1	1	1		3
9–10									1	4	1		6
10–11										2			2
11–12											2		2
12–13													0
13–14											1	1	2
Totals	3	10	13	26	35	51	47	48	37	23	6	1	300

FIG. 87.— *Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative. Early Champion*

r = .859 ± .010

Series 1238

	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	100–105	Totals
0– 1	3		4	2	1											10
1– 2		5	8	4	3	1			1							22
2– 3			5	14	5	1	1									26
3– 4			1	3	12	7	2	2								27
4– 5				3	8	8	2	1								22
5– 6					4	10	4									18
6– 7					2	6	3	4	1	1						17
7– 8						2	13	8	1	1						25
8– 9						3	9	6		2						20
9–10							1	10	4							15
10–11							3	8	2	1						14
11–12						1		6	7	1	1					16
12–13								1	5	3						9
13–14								2	5	1	2	2				12
14–15								2	3	3	2					10
15–16									2	2	2	1				7
16–17						1					1	1				3
17–18										3	2		1			6
18–19									1	1	1	2				5
19–20										1	4					5
20–21											2	1				3
21–22											1					1
22–23											1		1			2
23–24												1	1			2
24–25												1				1
27–28															1	1
28–29														1		1
Totals	3	5	18	26	35	40	38	50	32	20	19	9	3	1	1	300

FIG. 88.*—*Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative. Welcome*

r = .889 ± .008

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[*] See footnote to Fig 73, page 93.

Series 1257

	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	Totals
0– 1	1	3	10	12	8							34
1– 2				4	16	20	5	1				46
2– 3						23	25	7				55
3– 4						2	15	17	2	1		37
4– 5						1	3	20	8	1		33
5– 6							1	4	14	8	2	29
6– 7							1		9	11	2	23
7– 8									5	8	3	16
8– 9									3	7	1	11
9–10									1	4	5	10
10–11									1		3	4
11–12											1	1
12–13											1	1
Totals	1	3	10	16	24	46	50	49	43	40	18	300

FIG. 89.— *Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative. Sixty Day*

r = .875 ± .009

When average yield of culm per plant is correlated with average number of kernels per culm of plant (Figs. 90 to 93), the difference in the coefficients is slightly greater. The highest, .985 ± .001 for series 1238, is .051 ± .005 higher than the lowest, .934 ± .005 for series 1219. This extreme difference, although small, is more than ten times its probable error.

Series 1200

	0– 4	4– 8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	68–72	72–76	76–80	Totals
0– 1	3	3																	6
1– 2		9	17	3															29
2– 3			10	13	1														24
3– 4				8	14	2	1												25
4– 5				2	20	12	4												38
5– 6					4	12	11	6	2										35
6– 7						4	10	17	1										32
7– 8						1	3	9	6	2									21
8– 9								11	7	4		1	1						24
9–10								1	7	6	2	1							17
10–11									4	5	5	1							15
11–12										5	3	1	1						10
12–13												4	4						8
13–14												3	1						4
14–15												1	1	1					3
15–16													1						1
16–17													1		1				2
17–18															1				1
18–19															1				1
19–20																2	1	1	4
Totals	3	12	27	26	39	31	29	44	27	22	10	12	10	1	3	2	1	1	300

FIG. 90.*— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative. Great American*

r = .959 ± .003

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* See footnote to Fig. 73, page 93.

Series 1219

	0– 4	4– 8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	68–72	72–76	76–80	80–84	84–88	88–92	92–96	Totals
0– 1	8	17	11	5																					41
1– 2		1	18	18	15	7																			59
2– 3				6	5	19	20	6	3																59
3– 4						9	1	6	12	6	4														38
4– 5							4	3	5	12	5	4		1	1										35
5– 6									1	4	2	4	2	8	2	1									24
6– 7										3	1	3	5	2	5	1									20
7– 8												1	1	2	3	1		1							9
8– 9													1		1	1									3
9–10															2	1	2		1						6
10–11															1					1					2
11–12																		1					1		2
12–13																									0
13–14																				1				1	2
Totals	8	18	29	29	20	35	25	15	21	25	12	12	9	13	15	5	2	2	1	2	0	0	1	1	300

FIG. 91.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative. Early Champion*

$r = .934 \pm .005$

Series 1238	0– 4	4– 8	8– 12	12– 16	16– 20	20– 24	24– 28	28– 32	32– 36	36– 40	40– 44	44– 48	48– 52	52– 56	56– 60	60– 64	64– 68	68– 72	72– 76	76– 80	80– 84	84– 88	88– 92	92– 96	96–100	Totals
0– 1	6	4																								10
1– 2	1	14	7																							22
2– 3			22	4																						26
3– 4			4	20	3																					27
4– 5				3	13	6																				22
5– 6					6	6	5		1																	18
6– 7					2	9	5		1																	17
7– 8						1	13	7	1	2	1															25
8– 9							5	8	5	2																20
9–10								5	8	1	1															15
10–11									5	5	3	1														14
11–12									1	7	7	1														16
12–13										1	5	2	1													9
13–14											1	3	6	2												12
14–15												1	7	2												10
15–16													1	4	1		1									7
16–17															2	1										3
17–18															1		4	1								6
18–19																2	1	1		1						5
19–20																	3	2								5
20–21																		1	2							3
21–22																			1							1
22–23																			2							2
23–24																					1	1				2
24–25																					1					1
25–26																										0
26–27																										0
27–28																								1		1
28–29																									1	1
Totals	7	18	33	27	24	22	28	20	22	18	18	8	15	8	4	3	9	5	5	1	2	1	0	1	1	300

FIG. 92.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative. Welcome*

r = .985 ± .001

Series 1257

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	68–72	72–76	76–80	Totals
0– 1	12	17	5																		34
1– 2		2	17	18	8	1															46
2– 3			1	9	24	14	6		1												55
3– 4						9	18	5	3	2											37
4– 5						3	4	10	9	5		2									33
5– 6									8	9	9	1	1	1							29
6– 7										2	11	5	3		2						23
7– 8											2	1	6	3	4						16
8– 9													2	3	6						11
9–10															6	2		1	1		10
10–11																2	2				4
11–12																				1	1
12–13																				1	1
Totals	12	19	23	27	32	27	28	15	21	18	22	9	12	7	18	4	2	1	1	2	300

FIG. 93.—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative. Sixty Day*
r = .965 ± .003

For average yield of culm per plant correlated with average number of spikelets per culm of plant (Figs. 94 to 97), there is an extreme difference of .069 ± .006 between the coefficients. Series 1238 is again highest, with .985 ± .001, and series 1219 is again lowest, with .916 ± .006. The difference is more than eleven times its probable error.

For average yield of culm per plant correlated with average weight of straw per culm of plant (Figs. 98 to 101), the highest coefficient is .944 ± .004, again for series 1238, and the lowest is .818 ± .013 for series 1200. The difference here, .126 ± .014, is nine times its probable error.

Series 1200

	0– 2	2– 4	4– 6	6– 8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	Totals
0– 1	2	4																				6
1– 2		6	18	4	1																	29
2– 3			7	15	2																	24
3– 4				5	14	4	1	1														25
4– 5					17	14	6	1														38
5– 6					1	11	12	7	4													35
6– 7						2	7	12	9	2												32
7– 8							2	8	6	5												21
8– 9								3	13	4	2	1	1									24
9–10								1	2	9	3	1	1									17
10–11										4	6	4	1									15
11–12											6	2	1		1							10
12–13												2	4	2								8
13–14												1	1	2								4
14–15														3								3
15–16														1								1
16–17														1		1						2
17–18																	1					1
18–19																	1					1
19–20																			1	1	2	4
Totals	2	10	25	24	35	31	28	33	34	24	17	11	9	9	1	1	2	0	1	1	2	300

FIG. 94.—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative. Great American*

r = .961 ± .003

Series 1219

	0– 2	2– 4	4– 6	6– 8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	42–44	44–46	46–48	48–50	Totals
0– 1	2	8	14	7	9	1																				41
1– 2			4	11	14	17	11	1	1																	59
2– 3				1	3	10	13	16	10	4	2															59
3– 4						3	2	6	4	6	10	5	2													38
4– 5							2	1	2	5	11	5	4	3	2											35
5– 6											5	1	4	4	5	4		1								24
6– 7									1	1	1	2	4	3	1	2	2	2		1						20
7– 8													1	2	2	1	2			1						9
8– 9																2		1								3
9–10																1	1	2	1			1				6
10–11																1					1					2
11–12																					1			1		2
12–13																										0
13–14																					1				1	2
Totals	2	8	18	19	26	31	28	24	18	16	29	13	15	12	10	11	5	6	1	2	3	1	0	1	1	300

FIG. 95.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative. Early Champion*

$r = .916 \pm .006$

	0– 2	2– 4	4– 6	6– 8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	42–44	44–46	46–48	48–50	50–52	52–54	54–56	Totals
0– 1	6	3	1																										10
1– 2		13	9																										22
2– 3			16	9	1																								26
3– 4			2	16	8	1																							27
4– 5				1	11	8	2																						22
5– 6					3	7	5	2	1																				18
6– 7					1	6	8	1	1																				17
7– 8							7	9	6	1	2																		25
8– 9							1	6	9	3	1																		20
9–10									4	8	2	1																	15
10–11									2	3	8		1																14
11–12										1	5	8	2																16
12–13											1	2	6																9
13–14												1	1	6	4														12
14–15													2	6	1	1													10
15–16														1	1	3	1	1											7
16–17															1		2												3
17–18																	1		2	2	1								6
18–19																		2	2				1						5
19–20																		1	2	2									5
20–21																					3								3
21–22																					1								1
22–23																					1	1							2
23–24																							1	1					2
24–25																						1							1
27–28																										1			1
28–29																												1	1
Totals	6	16	28	26	24	22	23	18	23	16	19	12	12	13	7	4	4	4	6	4	6	2	2	1	0	1	0	1	300

FIG. 96.*—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative. We'come*

r = .985 ± .001

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*See footnote to Fig. 73, page 93.

Series 1257

	0– 2	2– 4	4– 6	6– 8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	Totals
0– 1	12	16	6																			34
1– 2		1	16	18	8	3																46
2– 3				12	25	12	6															55
3– 4					4	11	11	8	1	2												37
4– 5						3	7	9	7	4	1	1	1									33
5– 6								3	9	7	7	1	1		1							29
6– 7									2	3	11	2	1	2	2							23
7– 8										2	2		7	2	2	1						16
8– 9													1	5	3	2						11
9–10													1		4	3	1			1		10
10–11														1		1	1	1				4
11–12																					1	1
12–13																				1		1
Totals	12	17	22	30	37	29	24	20	19	18	21	4	12	10	12	7	2	1	0	2	1	300

FIG. 97.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative. Sixty Day*

r = .954 ± .003

	2– 3	3– 4	4– 5	5– 6	6– 7	7– 8	8– 9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	48–49	Totals
0– 1	4	1	1																					6
1– 2	4	11	10	1	2		1																	29
2– 3		4	13	5	2																			24
3– 4			4	16	2	1	1			1														25
4– 5			1	6	22	7	2																	38
5– 6				2	7	13	6	3	2		2													35
6– 7					1	12	9	8	1			1												32
7– 8						1	7	8	3	1		1												21
8– 9						1	1	7	9	4	2													24
9–10								1	7	7			2											17
10–11								1	2	5	3	3			1									15
11–12										4	1	2			2	1								10
12–13												4		2					1				1	8
13–14												1	2	1										4
14–15													2				1							3
15–16														1										1
16–17														1		1								2
17–18																	1							1
18–19																			1					1
19–20																			1		1	2		4
Totals	8	16	29	30	36	35	27	28	24	22	8	12	6	5	3	2	2	0	3	0	1	2	1	300

FIG. 98.*— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative. Great American*

$r = .818 \pm .013$

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* See footnote to Fig. 73, page 93.

Series 1219

	0– 1	1– 2	2– 3	3– 4	4– 5	5– 6	6– 7	7– 8	8– 9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	Totals
0– 1	4	18	17	2													41
1– 2		3	26	21	8		1										59
2– 3			1	23	24	11											59
3– 4				1	6	23	3	4	1								38
4– 5					2	8	19	5	1								35
5– 6						2	5	8	5	3	1						24
6– 7								7	6	5	1				1		20
7– 8							1	2	3	2	1						9
8– 9									1	1	1						3
9–10											3	2	1				6
10–11												1	1				2
11–12														2			2
12–13																	0
13–14																2	2
Totals	4	21	44	47	40	44	29	26	17	11	7	3	2	2	1	2	300

FIG. 99.—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative. Early Champion*

r = .933 ± .005

	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–32	Totals
0– 1	2	2	2	4																												10
1– 2	1	3	11	5	1						1																					22
2– 3			7	13	6																											26
3– 4			1	5	6	9	5	1																								27
4– 5				3	5	7	3	2	1							1																22
5– 6					2	9	5	2																								18
6– 7					1	3	5	6	1		1																					17
7– 8						1	8	9	4		1			1			1															25
8– 9							2	4	7	4		1		2																		20
9–10								1	3	4	3	3			1																	15
10–11							1	1		2	6	2	2																			14
11–12									1	1	4	4	2	4																		16
12–13											1		3	4	1																	9
13–14													4	3	3	1	1															12
14–15												2	3		4			1														10
15–16														1	3	1	1	1														7
16–17														1		1	1															3
17–18																2	1		1	1	1											6
18–19																1	1		1			1		1								5
19–20															1		1	1	1		1											5
20–21																			1	2												3
21–22																				1												1
22–23																					1				1							2
23–24																			1					1								2
24–25																							1									1
25–26																																0
26–27																																0
27–28																															1	1
28–29																											1					1
Totals	3	5	21	30	21	29	29	26	17	11	17	12	14	16	13	7	7	3	5	4	3	1	1	2	1	0	1	0	0	0	1	300

FIG. 100.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative. Welcome*

$r = .944 \pm .004$

Series 1257

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	Totals
0– 1	2	23	9																	34
1– 2		7	23	11	4			1												46
2– 3			5	36	13	1														55
3– 4			1	5	18	12			1											37
4– 5					4	20	6	2		1										33
5– 6						4	10	9	4		1				1					29
6– 7						1	5	6	6	4	1									23
7– 8							1	6	4	3		1		1						16
8– 9									4	3	3		1							11
9–10											6	2	2							10
10–11											2	2								4
11–12																	1			1
12–13																			1	1
Totals	2	30	38	52	39	38	22	24	19	11	13	5	3	1	1	0	1	0	1	300

Fig. 101.—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative. Sixty Day*
r = .925 ± .006

For average yield of culm per plant and average weight of kernels per plant (Figs. 102 to 105), the highest coefficient is .686 ± .021 for series 1200, and the lowest is .539 ± .028 for series 1219. The difference here is .147 ± .035, which amounts to about four times its probable error.

Series 1200

	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–32	32–33	Totals
0– 1	1				2		1		1								1							6
1– 2	1	2	2	3	3	4	1	5	2	2		1	2		1									29
2– 3					2		6		3	3	5		1	2		1							1	24
3– 4				1	1		1	2		5	2	3	6	3				1						25
4– 5							1	1	3	3	5	5	7	7	1	2	1		2					38
5– 6							2	2	2	6	1	3	8	2	3	3		2		1				35
6– 7									1	1	8	5	3	1	4	4	2	2		1				32
7– 8									1	1	1	2	4	3	3	2			3	1				21
8– 9								2				2	2	2	1	6	3	2	2	1	1			24
9–10												1	2	1	2	3	4		1	3				17
10–11													1	2	1	1	3	3	2	1	1			15
11–12													1		1		1	1	2	2	1	1		10
12–13															2	2	1	1	2					8
13–14																	1	1	1		1			4
14–15																		1		2				3
15–16																					1			1
16–17																		1				1		2
17–18																			1					1
18–19																						1		1
19–20															1		1	2						4
Totals	2	2	2	4	8	4	12	12	13	21	22	22	37	23	20	24	18	17	16	12	5	3	1	300

FIG. 102.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative. Great American*

r = .686 ± .021

Series 1219

	2- 3	3- 4	4- 5	5- 6	6- 7	7- 8	8- 9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	Totals
0- 1	1	4	4	5	8	6	3	4	1	2				2	1			41
1- 2				1	2	9	9	10	10	7	4	2	4	1				59
2- 3						2	8	15	9	11	3	3	2	3		1	2	59
3- 4							5	8	8	5	5	2	3	1	1			38
4- 5						1	1	1	12	5	6	3	2		1	2	1	35
5- 6								6	7	2	5	2	1	1				24
6- 7									4	3	5	1	2	4		1		20
7- 8										2	2	4		1				9
8- 9												1	1		1			3
9-10											1		2	2	1			6
10-11												1			1			2
11-12											1				1			2
12-13																		0
13-14												1			1			2
Totals	1	4	4	6	10	18	26	44	51	37	32	20	17	15	8	4	3	300

FIG. 103.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative.* ***Early Champion***

$r = .539 \pm .028$

Series 1238

	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	27–28	28–29	29–30	30–31	31–32	32–33	33–34	Totals
0–1	1					1			2		1	2	1	1					1									10
1–2						1			1	1	4	3	4	1	2	1	1		1	1		1						22
2–3											1	1	3	4		3	6	4	2	2								26
3–4											1		1	2	3	1	3	6	3	3	2			2				27
4–5												1	1	2	1	2	4	3	2	2	2	1			1			22
5–6									1					3	1	3	4		2	1	1		1	1				18
6–7													1					3	1	4	2	2	1	2			1	17
7–8												1		2	1			3	4	5	3	3		2	1			25
8–9																	3	2		2	4	4	4		1			20
9–10																	1		2	3	3	1	2	2	1			15
10–11																1		1	1	3		3	3	2				14
11–12																			2		5	4	3	2				16
12–13																				1	2	2	2	1		1		9
13–14																		1	3	4	1	2		1				12
14–15																				2	1	3	1	2	1			10
15–16																		1			4	1	1					7
16–17																			1			2						3
17–18																			1	3	1			1				6
18–19																	1				1	2	1					5
19–20																					1	2	2					5
20–21																					2		1					3
21–22																						1						1
22–23																							1	1				2
23–24																					1	1						2
24–25																							1					1
25–26																												0
26–27																												0
27–28																						1						1
28–29																						1						1
Totals	1	0	0	0	0	2	0	0	4	1	7	8	11	15	8	11	23	24	26	36	36	37	24	19	5	1	1	300

FIG. 104.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative.* *Welcome*

r = .596 ± .025

Series 1257

	5- 6	6- 7	7- 8	8- 9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	Totals
0- 1		2	7	5	1	8	3	1	2	3	1	1		34
1- 2	1		1	3	4	8	9	5	9	4		1	1	46
2- 3			1		1	4	15	14	5	7	5	1	2	55
3- 4					1	3	5	8	13	4	2	1		37
4- 5				1		2	2	9	3	3	8		5	33
5- 6						1	1	5	7	7	7	1		29
6- 7							2	2	4	7	3	4	1	23
7- 8								2	6	3	2	3		16
8- 9									2	5	3	1		11
9-10									1	2	2	5		10
10-11										1		1	2	4
11-12											1			1
12-13											1			1
Totals	1	2	9	9	7	26	37	46	52	46	35	19	11	300

FIG. 105.—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative. Sixty Day*

r = .563 ± .027

When the average weight of kernels per plant is correlated with the average height of plant (Figs. 106 to 109), the highest coefficient is again found in series 1200, where it is .654 ± .022. The lowest is again in series 1219, where it is .553 ± .027. The difference is here .101 ± .035, which appears rather large but in fact is less than three times its probable error.

Series 1200

	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Totals
10–11	1	1										2
11–12			1		1							2
12–13			1	1								2
13–14			2		1			1				4
14–15	1	4		2		1						8
15–16		2	1	1								4
16–17		1	2	5	2	2						12
17–18		1	4	2	2	1	2					12
18–19		1	1	5	2	3	1					13
19–20		1	2	3	6	6	3					21
20–21			3	3	9	6	1					22
21–22			1	1	8	5	7					22
22–23				8	8	14	4	3				37
23–24			1	1	7	6	6	2				23
24–25		1			2	6	5	3	2	1		20
25–26		1		1	3	6	6	5	2			24
26–27		1		1		3	5	7			1	18
27–28				1		2	7	1	4	2		17
28–29					1	5	1	7	1	1		16
29–30						2	6	2	1	1		12
30–31							1	4				5
31–32								1	2			3
32–33					1							1
Totals	2	14	19	35	53	68	55	36	12	5	1	300

FIG. 106.—*Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative. Great American*

r = .654 ± .022

Series 1219

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Totals
2– 3	1												1
3– 4	2	2											4
4– 5		1	3										4
5– 6		1	3		1		1						6
6– 7		3	2	3	2								10
7– 8		2	1	5	3	4	2		1				18
8– 9			1	3	7	8	2	2	2	1			26
9–10			2	1	7	10	13	6	4	1			44
10–11			1	4	3	11	9	11	9	3			51
11–12				7	2	6	9	7	3	3			37
12–13					3	3	3	11	7	4	1		32
13–14					3	2	3	3	3	4	2		20
14–15				1	2	3	2	3	3	2	1		17
15–16		1		1	1	2	1	2	3	3	1		15
16–17				1		1		1	1	2	1	1	8
17–18					1		1	1	1				4
18–19						1	1	1					3
Totals	3	10	13	26	35	51	47	48	37	23	6	1	300

FIG. 107.—*Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative. Early Champion*

$r = .553 \pm .027$

Series 1238

	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	100–105	Totals
7– 8			1													1
12–13				2												2
15–16	1		2				1									4
16–17			1													1
17–18		1	1	3	1				1							7
18–19	1	1	1	3	1		1									8
19–20		1	2	3	3	1		1								11
20–21	1	1	2	2	4	2	1	1		1						15
21–22			1	1	3	1	2									8
22–23		1		1	3	3	2			1						11
23–24			2	3	3	9	3	1	1			1				23
24–25				4	4	3	5	4	2	1	1					24
25–26			2	2	5	5	1	5	3	2	1					26
26–27			2	1	2	4	6	12	3	1	3	1	1			36
27–28					1	7	3	7	5	5	4	4				36
28–29			1	1	1	2	4	8	6	4	5	2	1	1	1	37
29–30						1	5	5	4	4	3	1	1			24
30–31					3	2	2	4	6	1	1					19
31–32					1		1	2			1					5
32–33									1							1
33–34							1									1
Totals	3	5	18	26	35	40	38	50	32	20	19	9	3	1	1	300

FIG. 108.*— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative. Welcome*

r = .575 ± .026

Series 1257

	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	Totals
5– 6						1						1
6– 7			2									2
7– 8	1		2	3	1		2					9
8– 9		3		2	2		2					9
9–10					4	1		2				7
10–11			3	5	3	5	5	3	1		1	26
11–12				2	7	8	13	3	4			37
12–13				1	1	15	8	11	8	2		46
13–14			1	1	3	6	9	14	7	10	1	52
14–15			1	2	1	5	6	4	11	13	3	46
15–16			1			2	3	7	6	9	7	35
16–17					1	1	1	2	3	6	5	19
17–18					1	2	1	3	3		1	11
Totals	1	3	10	16	24	46	50	49	43	40	18	300

FIG. 109.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative. Sixty Day*

r = .577 ± .026

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* See footnote to Fig. 73, page 93.

For average weight of kernels per plant correlated with average number of kernels per culm of plant (Figs. 110 to 113), the highest coefficient is .524 ± .028 for series 1200, and the lowest is .340 ± .034 for series 1219. The difference here is .184 ± .044, which is a little more than four times its probable error.

Series 1200

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	68–72	72–76	76–80	Totals
10–11		1	1																2
11–12			1	1															2
12–13			2																2
13–14			2	1			1												4
14–15	1	2	2	2		1													8
15–16			3	1															4
16–17	1		1	5	1	1	1		2										12
17–18		3	2		2		1	2				1	1						12
18–19		2	4			2	1	3		1									13
19–20		1	3	1	5	2	5	2	1	1									21
20–21			1	4	4	3	1	8	1										22
21–22			1	2	4	2	3	5	2	2		1							22
22–23		2		6	7	6	3	5	3	1	2	1	1						37
23–24			2		8	4	1	2	2	2	2								23
24–25		1			1	4	3	3	1	2	1	1	2					1	20
25–26			1		4	1	4	4	5	2	1	1	1						24
26–27	1				1	1	1	3	3	3	2		2				1		18
27–28				1	1	2	1	2		3	1	2		1	1	2			17
28–29				2			3	2	3	1	1	3			1				16
29–30					1	2		2	3	2		1	1						12
30–31								1	1	1		1	1						5
31–32										1			1		1				3
32–33			1																1
Totals	3	12	27	26	39	31	29	44	27	22	10	12	10	1	3	2	1	1	300

FIG. 110.*—*Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative. Great American*

r = .524 ± .028

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* See footnote to Fig. 73, page 93.

Series 1219

	0– 4	4– 8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	68–72	72–76	76–80	80–84	84–88	88–92	92–96	Totals
2– 3	1																								1
3– 4	2	2																							4
4– 5	1	2		1																					4
5– 6		2	2	1		1																			6
6– 7	1	3	2	4																					10
7– 8	2		3	5	3	2		1	1						1										18
8– 9		1	2	2	4	3	3	3	3	2	2			1											26
9–10		2	2	4	5	8	6	2	2	4	2	1		4	1	1									44
10–11		1	7	1	3	2	6	2	6	4	5	3	2	4	5										51
11–12		2	4	2	2	5	6	1	3	5	1	1	1	1		2		1							37
12–13			2	2		5		3	3	4	1	3	3	1	3				1				1		32
13–14			2		2	3		2	1	2			2	2	1	1				1				1	20
14–15		1	2	2		4		1	1	1		2			1		2								17
15–16	1	1	1	2	1	1			1	2	1	2			1	1									15
16–17		1				1	1						1		2			1		1					8
17–18				1			2			1															4
18–19				2			1																		3
Totals	8	18	29	29	20	35	25	15	21	25	12	12	9	13	15	5	2	2	1	2	0	0	1	1	300

FIG. 111.—*Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative. Early Champion*

$r = .340 \pm .034$

Series 1238

	0– 4	4– 8	8– 12	12– 16	16– 20	20– 24	24– 28	28– 32	32– 36	36– 40	40– 44	44– 48	48– 52	52– 56	56– 60	60– 64	64– 68	68– 72	72– 76	76– 80	80– 84	84– 88	88– 92	92– 96	96–100	Totals
7- 8		1																								1
12–13		2																								2
15–16	2		1						1																	4
16–17		1																								1
17–18		3	2	1	1																					7
18–19	1	2	3			1					1															8
19–20	1	3	3	2		1			1																	11
20–21	1	1	3	2	2	1	3			2																15
21–22		1	1	2	1	1	1		1																	8
22–23		1	2	2	1	3	1					1														11
23–24		1	6	3	3	5			2	1	1									1						23
24–25			4	6	3		3	3	1	1	1			1			1									24
25–26	1	1	4	1	4		2	3	1	1	2	1	2	1		1		1								26
26–27		1	2	3	3	4	4	2	4	2	1		5	2			3									36
27–28			1	2	2	1	4	3	4	1	4	3	1	3	1		1	2	2			1				36
28–29	1			1		2	5	3		5	4	2	3	1	2	1	2	1	1		1			1	1	37
29–30					1	1	2	3	4	3	2		2			1	2	1	1		1					24
30–31			1	1	2	1	2	2	3	1	2		2		1				1							19
31–32				1		1	1	1				1														5
32–33										1																1
33–34					1																					1
Totals	7	18	33	27	24	22	28	20	22	18	18	8	15	8	4	3	9	5	5	1	2	1	0	1	1	300

FIG. 112.*—*Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative.* **Welcome**

$r = .494 \pm .029$

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* See footnote to Fig. 73, page 93.

Series 1257

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	68–72	72–76	76–80	Totals
5– 6					1																1
6– 7		1	1																		2
7– 8	2	3	2		1				1												9
8– 9	3	1	1	2		1						1									9
9–10		1	2		2		1			1											7
10–11	3	4	3	2	4		4		2	2		1		1							26
11–12		3	5	4	7	7	4	1	2	1			1		2						37
12–13		1	2	3	9	5	5	4	5	3	5		2		2						46
13–14		2	4	6	2	7	7	2	2	2	4	4	2	3	4				1		52
14–15	2	2	1	4	5	2	2	2	3	5	5	2	3	2	3	1	1	1			46
15–16	1			4	1	1	3	6	5	2	3		3	1	2	1				2	35
16–17	1		2			1			1	1	5	1	1		5		1				19
17–18		1		2		3	2			1						2					11
Totals	12	19	23	27	32	27	28	15	21	18	22	9	12	7	18	4	2	1	1	2	300

FIG. 113.—*Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative. Sixty Day*

r = .415 ± .032

There are some slight differences observable in the coefficients here determined, which may be mentioned as possibly having some varietal significance. The highest correlation coefficient is found in series 1238 in four out of seven comparisons, and in the remaining three cases it is high. In no case is it the lowest. In three out of seven cases, series 1200 has the highest coefficient, and once it has the lowest coefficient. Series 1219 has the lowest coefficient in six out of seven cases. One would expect, then, to find in the Welcome variety of oats relatively high correlation between all characters here used in correlation studies. In the Great American variety there is relatively high correlation when average weight of kernels per plant is one of the characters considered, and relatively low when yield is correlated with weight of straw, although the coefficient is very high in the latter case. In the Early Champion variety relatively low correlations may be expected when practically all the characters here concerned are correlated, but especially when average weight of kernels is a factor. The coefficients themselves, however, are high. The Sixty Day variety seems to occupy an intermediate position as regards correlation of the characters under consideration.

The differences between the coefficients of correlation for the same characters determined for the different varieties are assembled in Table 6:

TABLE 6. Summary of Differences in Correlation Coefficients for the Same Characters in the Four Varieties Compared

Characters correlated	Difference	Difference / P. E. difference
Yield and height	.030 ± .013	2.3
Yield and number of kernels	.051 ± .005	10.2
Yield and number of spikelets	.069 ± .006	11.5
Yield and weight of straw	.126 ± .014	9.0
Yield and weight of kernels	.147 ± .035	4.2
Weight of kernels and height	.101 ± .035	2.9
Weight of kernels and number of kernels	.184 ± .044	4.2

The largest absolute difference between the correlation coefficients for the different varieties occurs in the correlation between average weight of kernels and average number of kernels per culm, where it is .184 ± .044. The relative difference here, as indicated by the result obtained by dividing the difference by its probable error, is 4.2 times the probable error. The second largest absolute difference is when yield is correlated with average weight of kernels, the difference here being .147 ± .035, which is again 4.2 times the probable error. The third largest absolute difference is when yield is correlated with average weight of straw, the difference here being .126 ± .014, which is just nine times the probable error. The only other difference above .10 is that existing when average weight of kernels is correlated with height, which is .101 ± .035, this being 2.9 times the probable error. The difference in the correlations between yield and number of kernels is not large in absolute amount, being but .051 ± .005, but, owing to the small probable error, it amounts to 10.2 times the latter. The difference in the correlation between yield and number of spikelets is .069 ± .006, which is 11.5 times its probable error. This absolute difference is again small, but the relative difference is large because of its small probable error. The difference between the correlations of yield and height is both absolutely and relatively small, being .030 ± .013.

The greatest absolute differences, then, are seen to be found when weight of straw and average weight of kernels are correlated with other characters, while the greatest relative differences are found when yield is correlated with weight of straw and number of kernels and of spikelets.

The coefficients here reported are usually fairly close together for the different varieties. No striking differences occur, such as exist between the results of Waldron and those of the writer. Since, however, the variety used by Waldron is not here concerned, it cannot be said that the difference in his results was not due to varietal causes. Some differences occur in these results which are evidently due to varietal causes, hence we must conclude that differences in correlation coefficients may result from the use of different varieties.

Comparison of biometrical constants determined for oat plants grown in hills and in drills

Some statistical work has been done on cereals in which the plants used were grown a fixed and uniform distance apart, the plants each being thus allowed the same amount of space in which to grow. Other work has been done with plants that were grown in rows, in which the grain was drilled in the ordinary method of seeding. The work of Roberts (1911) and of Myers (1911) on wheat was with plants grown in hills several inches apart. The work of Noll (1911) on wheat was with plants or culms that grew in drilled rows. The work of Atkinson (1912) was with wheat that was seeded broadcast in plats, the same quantity of different varieties being used. The work of Waldron (1910) on oats was with plants from drilled rows.

What differences, if any, exist in the constants determined for plants grown in these ways? In which case is there greater variability, if difference exists? In which is there greater correlation, if difference exists?

In order to test the effect of growing plants in separate hills and in drilled rows on the biometrical results, the following plantings were made:

Sixty Day selection, pure line 62–II–6–3, and Early Champion selection, pure line 137–6, were each sown in drills, fifteen grams being sown uniformly in eighteen-foot rows, the rows being one foot apart. Separate kernels of the two lines were also planted in hills three inches apart in eighteen-foot rows, the rows again being one foot apart. All plants were harvested separately, measured, and the constants determined. The conditions may be summarized as follows:

Series	Variety	Method of sowing
1219	Early Champion	In drills
1221	Early Champion	In hills
1257	Sixty Day	In drills
1259	Sixty Day	In hills

The date of harvesting (pulling), the varieties, and the figures showing thickness of stand are:

Series	Variety	Harvested	Thickness of stand
1219	Early Champion	July 24, 1911	600 plants in 18 feet = 33.3 plants per foot
1221	Early Champion	August 4, 1911	3 inches apart in rows
1257	Sixty Day	July 24, 1911	600 plants in 18 feet = 33.3 plants per foot
1259	Sixty Day	August 4, 1911	3 inches apart in rows

The numbers of culms produced by the plants grown under the different conditions are:

Series	Method of growing	Number of culms per plant								
		1	2	3	4	5	6	7	8	9
1219	In drills	295	5	...	..	..	..	..	..	...
1221	In hills	41	52	144	39	18	4	1	1	...
1257	In drills	288	11	1	..	..	..	..	..	...
1259	In hills	37	51	103	58	39	8	1	2	1

The number of culms per plant is thus seen to be reduced practically to the minimum by growing the plants in drilled rows.

The means (M), standard deviations (σ), and coefficients of variability (C) for the plants grown from these sowings are given in Table 7:

TABLE 7. MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY, SERIES 1219, 1221, 1257, 1259.* VARIETIES IN HILLS AND IN DRILLS

Average yield of culm per plant, in decigrams				
	1219	1221	1257	1259
M....	3.433 ± .096	7.890 ± .118	3.950 ± .102	9.087 ± .124
σ....	2.473 ± .068	3.034 ± .083	2.628 ± .072	3.186 ± .088
C.....	72.04 ± 2.82	38.45 ± 1.20	66.53 ± 2.51	35.06 ± 1.08
Average height of plant, in centimeters				
	1219	1221	1257	1259
M....	55.850 ± .432	58.683 ± .290	49.566 ± .416	51.916 ± .252
σ....	11.114 ± .306	7.462 ± .205	10.703 ± .294	6.486 ± .178
C.....	19.90 ± .57	12.72 ± .36	21.59 ± .62	12.49 ± .35
Average number of kernels per culm of plant				
	1219	1221	1257	1259
M....	29.293 ± .695	55.293 ± .737	28.440 ± .663	55.480 ± .700
σ....	17.867 ± .491	18.942 ± .521	17.054 ± .469	17.992 ± .495
C.....	60.99 ± 2.21	34.26 ± 1.05	59.96 ± 2.16	32.43 ± .98
Average number of spikelets per culm of plant				
	1219	1221	1257	1259
M....	17.160 ± .359	31.427 ± .393	14.000 ± .330	28.260 ± .357
σ....	9.230 ± .254	10.110 ± .278	8.475 ± .233	9.190 ± .253
C.....	53.79 ± 1.86	32.17 ± .97	60.54 ± 2.19	32.52 ± .98
Average weight of straw per culm of plant, in decigrams				
	1219	1221	1257	1259
M....	5.227 ± .107	10.190 ± .129	5.303 ± .116	9.423 ± .117
σ....	2.763 ± .076	3.317 ± .091	2.977 ± .082	2.997 ± .082
C.....	52.86 ± 1.81	32.55 ± .99	56.14 ± 1.97	31.81 ± .96
Average weight of kernels per plant, in milligrams				
	1219	1221	1257	1259
M....	10.913 ± .115	14.080 ± .066	13.067 ± .093	16.163 ± .063
σ....	2.967 ± .082	1.684 ± .046	2.400 ± .066	1.632 ± .045
C.....	27.19 ± .80	11.96 ± .33	18.37 ± .52	10.10 ± .28

* 1219, Early Champion in drills
1221, Early Champion in hills
1257, Sixty Day in drills
1259, Sixty Day in hills

Comparing first the means for average yield of culm per plant in decigrams, it is seen that these are, respectively, 3.433 ± .096 for series 1219; 7.890 ± .118 for series 1221; 3.950 ± .102 for series 1257; and 9.087 ± .124 for series 1259. It is thus clear that both varieties agree in having a greater mean for yield of culm when the plants are grown in hills than when grown in drills. The standard deviations are likewise greater for the plants grown in hills. There is, then, a larger yield per culm and a greater variability in yield per culm when plants are grown in hills than when they are grown in drills.

In the average height of plant in centimeters, the means are 55.850 ± .432 for series 1219; 58.683 ± .290 for series 1221; 49.566 ± .416 for series 1257; and 51.916 ± .252 for series 1259. The plants grown in hills, therefore, are on the average slightly taller than those grown in drills. As regards the standard deviations, conditions are reversed from what they were in average yield. For series 1219 the standard deviation is 11.114 ± .306, for series 1221 it is 7.462 ± .205, for series 1257 it is 10.703 ± .294, and for series 1259 it is 6.486 ± .178. It is thus seen that there is greater variability for average height in plants grown in drills than in plants grown in hills.

The means for average number of kernels per culm of plant are about twice as high in series 1221 and series 1259 as in series 1219 and series 1257, respectively. The standard deviations are slightly greater, also, for series 1221 and series 1259. These results indicate that culms grown on plants in hills produce nearly twice as many kernels as do culms grown on plants in drills, but that the variation is about the same.

The means for average number of spikelets per culm of plant are about twice as high in series 1221 and series 1259 as in series 1219 and series 1257, respectively. The standard deviations also are slightly greater in the two former series. This indicates that there are about twice as many spikelets produced on culms of plants grown in hills as on those grown in drills, but that the variation in number of spikelets on such culms is only slightly greater.

For average weight of straw per culm of plant in decigrams, the means for series 1221 and series 1259 are again nearly twice as high as for series 1219 and series 1257, respectively, and again the standard deviations are greater for the two former series. About twice as much straw per culm is therefore produced by plants growing in hills as compared with those grown in drills, and the variability in this character is also somewhat greater for plants grown in hills.

For average weight of kernels per plant in milligrams, the means are a little greater for series 1221 and series 1259 than for series 1219 and series 1257, respectively. The standard deviations here are higher for series 1219 and series 1257 than for their corresponding series. Larger kernels are therefore produced by plants grown in hills than by plants grown in drills, but the difference is not so great as that which occurs in any other character studied, with the exception of height of plant. The variability, however, in average kernel weight is greater in plants grown in drills, this being probably due to the fact that the kernels on the isolated plants have a more uniform opportunity to develop than do those in more crowded positions.

The results obtained for both varieties dealt with are identical in character.

Regarding all characters here studied of the plants grown under these two conditions, the means are greater in every case for the plants grown in hills than for those grown in drills. It is evident that the full development allowed to plants in more isolated positions is not permitted in more crowded positions. The least difference in the means occurs in average height of plant and average weight of kernels per plant. The crowded conditions do not hinder the growth in height and the development of the kernels so much as they hinder the increase in yield, number of kernels and of spikelets, and weight of straw.

There is greater variability in average yield of culm per plant and average weight of straw per culm of plant with plants grown in hills than with those grown in drills, but much less variability in average height of plant and average weight of kernels. The variability in number of kernels and number of spikelets is slightly greater for the plants grown in hills. The standard deviation is here taken as the index of variability.

Correlations

The correlations of the two varieties as grown in hills and in drills are given in Table 8:

TABLE 8. Correlations, Series 1219, 1221, 1257, 1259.* Varieties in Hills and in Drills

Average yield of culm per plant, in decigrams — Average height of plant, in centimeters

1219	r = .859 ± .010	1257	r = .875 ± .009
1221	r = .853 ± .011	1259	r = .788 ± .015

Average yield of culm per plant, in decigrams — Average number of kernels per culm of plant

1219	r = .934 ± .005	1257	r = .965 ± .003
1221	r = .952 ± .004	1259	r = .960 ± .003

Average yield of culm per plant, in decigrams — Average number of spikelets per culm of plant

1219	r = .916 ± .006	1257	r = .954 ± .003
1221	r = .931 ± .005	1259	r = .956 ± .003

Average yield of culm per plant, in decigrams — Average weight of straw per culm of plant, in decigrams

1219	r = .933 ± .005	1257	r = .925 ± .006
1221	r = .856 ± .010	1259	r = .787 ± .015

Average yield of culm per plant, in decigrams — Average weight of kernels per plant, in milligrams

1219	r = .539 ± .028	1257	r = .563 ± .027
1221	r = .493 ± .029	1259	r = .407 ± .032

Average weight of kernels per plant, in milligrams — Average height of plant, in centimeters

1219	r = .553 ± .027	1257	r = .577 ± .026
1221	r = .325 ± .035	1259	r = .186 ± .038

Average weight of kernels per plant, in milligrams — Average number of kernels per culm of plant

1219	r = .340 ± .034	1257	r = .415 ± .032
1221	r = .250 ± .036	1259	r = .186 ± .038

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* 1219, Early Champion in drills
1221, Early Champion in hills
1257, Sixty Day in drills
1259, Sixty Day in hills

Considering first the correlations between average yield of culm per plant and average height of plant (Figs. 87, 72, 89, 114), it is seen that the coefficients for series 1219 and series 1221 differ by only .006 ± .015, which is a negligible difference. In series 1257 the coefficient is .087 ± .017 greater than in series 1259. This is more than five times its probable error, and may be of significance.

Series 1259

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	Totals
0– 1	1									1
1– 2		1				1				2
2– 3		2	2	2	1					7
3– 4		1	3	3	1	1	1			10
4– 5			1	9	3	1				14
5– 6				8	7	5				20
6– 7				6	13	3	1			23
7– 8				2	17	14				33
8– 9				1	12	17	3			33
9–10					6	17	12		1	36
10–11					1	15	10	2		28
11–12						11	18	3		32
12–13					1	4	18	8		31
13–14						2	7	6		15
14–15						1	6	2		9
15–16						1	1	2		4
16–17							2			2
Totals	1	4	6	31	62	93	79	23	1	300

FIG. 114.— *Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative*

r = .788 ± .015

For average yield of culm per plant and average number of kernels per culm of plant (Figs. 91, 74, 93, 115), the coefficients are above .934 in every case, and are so close together in the same varieties for the two methods of sowing that no comment is necessary.

For average yield of culm per plant and average number of spikelets per culm of plant (Figs. 95, 76, 97, 116), the same relative conditions exist as in the preceding case. All the coefficients are above .916, and those for the same varieties are very near each other in value.

Series 1259

	0– 4	4– 8	8– 12	12– 16	16– 20	20– 24	24– 28	28– 32	32– 36	36– 40	40– 44	44– 48	48– 52	52– 56	56– 60	60– 64	64– 68	68– 72	72– 76	76– 80	80– 84	84– 88	88– 92	92– 96	96–100	Totals
0– 1	1																									1
1– 2				1	1																					2
2– 3			2		3	2																				7
3– 4						1	7	1	1																	10
4– 5						1	2	9	1	1																14
5– 6							1	3	7	8	1															20
6– 7								1	1	10	8	1	1			1										23
7– 8										2	8	11	7	4	1											33
8– 9											1	5	3	14	7	3										33
9–10													4	4	16	10	1	1								36
10–11														1	3	7	9	7	1							28
11–12															3	5	5	11	4	4						32
12–13																	3	9	7	11		1				31
13–14																			2	8	3	1	1			15
14–15																		1	1	2	3	1	1			9
15–16																			1			1		1	1	4
16–17																							1		1	2
Totals	1	0	2	1	4	4	10	14	10	21	18	17	15	23	30	26	18	29	16	25	6	4	3	1	2	300

FIG. 115.—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative*

r = .960 ± .003

Series 1259

	0– 2	2– 4	4– 6	6– 8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	42–44	44–46	46–48	48–50	Totals
0– 1	1																									1
1– 2				1	1																					2
2– 3			1	1	2	3																				7
3– 4							7	3																		10
4– 5						1	3	6	3	1																14
5– 6							2	1	5	8	4															20
6– 7								1	4	7	6	3		1		1										23
7– 8										2	5	11	8	4	3											33
8– 9											1	3	3	12	8	4	2									33
9–10													3	6	14	8	4	1								36
10–11														1	1	8	9	5	3	1						28
11–12															4	3	4	8	9	4						32
12–13																		10	5	9	6	1				31
13–14																			1	5	6	2	1			15
14–15																			2	2	2	2	1			9
15–16																				1			1		2	4
16–17																							1		1	2
Totals	1	0	1	2	3	4	12	11	12	18	16	17	14	24	30	24	19	24	20	22	14	5	4	0	3	300

FIG. 116.—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative*

r = .956 ± .003

For average yield of culm per plant and average weight of straw per culm of plant (Figs. 99, 78, 101, 117), the coefficient for series 1219 is .933 ± .005. This is greater than that for series 1221 by .077 ± .011. The coefficient for series 1257 is .925 ± .006. This is greater than that for series 1259 by .138 ± .016. The first of these differences is seven times its probable error; the second is a little less than nine times its probable error.

Series 1259

	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	20–21	28–29	Totals
0–1			1															1
1–2	1				1													2
2–3	2	1	2	1	1													7
3–4			5	1	3			1										10
4–5			4	8		1			1									14
5–6				3	8	5	2		2									20
6–7				5	7	6	2	2		1								23
7–8				1	6	13	9	1		1	2							33
8–9					2	8	11	8	1	3								33
9–10						2	12	10	6	2	2		1			1		36
10–11					1	2	1	8	6	8	2							28
11–12							1	4	6	10	8	2					1	32
12–13							1	2	4	5	11	6	2					31
13–14								1	1	3	6	2	1	1				15
14–15										1	5	3						9
15–16											1	1	2					4
16–17													1		1			2
Totals	3	1	12	19	29	37	39	37	27	34	37	14	7	1	1	1	1	300

FIG. 117.*—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative*

r = .787 ± .015

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* See footnote to Fig. 73, page 93.

For average yield of culm per plant correlated with average weight of kernels per plant (Figs. 103, 80, 105, 118), the coefficient for series 1219 is .539 ± .028, which is .046 ± .040 higher than that for series 1221. This difference is only a little more than the probable error. For series 1257 the coefficient is .563 ± .027, which is .156 ± .042 higher than that for series 1259. This difference is less than four times its probable error.

Series 1259

	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	Totals
0– 1			1										1
1– 2	1		1										2
2– 3				1	1		1	2		1	1		7
3– 4			1	4	1	2	1	1					10
4– 5				1	1	3	2	5	1		1		14
5– 6					1	5	5	4	2	2	1		20
6– 7			1	1		2	8	7	1	2	1		23
7– 8				1	5	2	7	12	3	1	2		33
8– 9					1	5	14	7	4	1	1		33
9–10						5	12	10	6	3			36
10–11					1	4	9	10	3		1		28
11–12						1	9	10	5	6	1		32
12–13						1	7	9	10	4			31
13–14						1		9	4	1			15
14–15								2	3	2	1	1	9
15–16							1	1		1	1		4
16–17								1		1			2
Totals	1	0	4	8	11	31	76	90	42	25	11	1	300

FIG. 118.—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative*
r = .407 ± .032

The average weight of kernels per plant correlated with average height of plant (Figs. 107, 82, 109, 119) shows a greater difference in the coefficients. For series 1219 the coefficient is .553 ± .027, and for series 1221 it is .325 ± .035. The difference is here .228 ± .044, which is about five times its probable error. For series 1257 the coefficient is .577 ± .026, and for series 1259 it is .186 ± .038, the difference here being .391 ± .046, which is eight and one half times its probable error. This difference is doubtless of significance.

Series 1259

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	Totals
9–10						1				1
10–11										0
11–12	1	1		1			1			4
12–13		1	2	2	2	1				8
13–14			1	2		7	1			11
14–15				6	4	12	6	3		31
15–16			2	5	18	28	17	5	1	76
16–17			1	11	19	24	24	11		90
17–18				1	9	11	18	3		42
18–19		1		2	6	5	10	1		25
19–20		1		1	4	4	1			11
20–21							1			1
Totals	1	4	6	31	62	93	79	23	1	300

FIG. 119.—*Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative*

r = .186 ± .038

For average weight of kernels per plant correlated with average number of kernels per culm of plant (Figs. 111, 84, 113, 120), the coefficient for series 1219 is .340 ± .034, and for series 1221 it is .250 ± .036, showing a difference of .090 ± .050, which is a little less than twice its probable error. Between series 1257 and series 1259 there is a difference of .229 ± .050, which is more than four times its probable error. This difference is probably significant.

Series 1259

	0– 4	4– 8	8– 12	12– 16	16– 20	20– 24	24– 28	28– 32	32– 36	36– 40	40– 44	44– 48	48– 52	52– 56	56– 60	60– 64	64– 68	68– 72	72– 76	76– 80	80– 84	84– 88	88– 92	92– 96	96–100	Totals
9–10					1																					1
10–11																										0
11–12	1			1					1							1										4
12–13						1	3	1		1			1	1												8
13–14						1	1	1		1			2	2	1	1			1							11
14–15							2	2	1	4	3		1	1	3	5	1	5		1		1	1			31
15–16					1		2	1	2	7	3	4	4	10	11	6	5	6	3	10					1	76
16–17					2	1		5	5	3	8	8	2	4	9	7	7	6	6	9	3	2	1	1	1	90
17–18							1	1	1	1	2	4	2	4	3	2	2	10	3	3	3					42
18–19			1					2		2	2		3		3	3	3	1	1	2		1	1			25
19–20			1			1	1	1		2		1		1		1			2							11
20–21																		1								1
Totals	1	0	2	1	4	4	10	14	10	21	18	17	15	23	30	26	18	29	16	25	6	4	3	1	2	300

FIG. 120.—*Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative*

$r = .186 \pm .038$

The correlations obtained between the several characters considered for plants of the same pure lines grown in hills and in drills do not in the main differ radically from each other for the two methods of planting. There are, however, some rather large differences in a few cases. The differences, as given in Table 9, range from .002 ± .004 to .391 ± .046. The latter is of considerable value and is doubtless significant. Five out of fourteen correlations have differences amounting to more than .10, and of these four are found in the Sixty Day variety. Only three have differences amounting to more than about five times their probable errors, and two of these are in Sixty Day, these two being when yield is correlated with weight of straw and when weight of kernels is correlated with average height; the third case is when average yield is correlated with weight of straw in Early Champion.

TABLE 9. SUMMARY OF DIFFERENCES IN CORRELATION COEFFICIENTS FOR THE SAME CHARACTERS IN THE SAME PURE LINES GROWN IN HILLS AND IN DRILLS

Characters correlated	Extreme difference		$\frac{\text{Difference}}{\text{P. E. difference}}$	
	1219 and 1221	1257 and 1259	1219 and 1221	1257 and 1259
Yield and height	.006 ± .015	.087 ± .017	0.4	5.1
Yield and number of kernels	.018 ± .006	.005 ± .004	3.0	1.2
Yield and number of spikelets	.015 ± .008	.002 ± .004	1.9	0.5
Yield and weight of straw	.077 ± .011	.138 ± .016	7.0	8.6
Yield and weight of kernels	.046 ± .040	.156 ± .042	1.1	3.7
Weight of kernels and height	.228 ± .044	.391 ± .046	5.2	8.5
Weight of kernels and number of kernels	.090 ± .050	.229 ± .050	1.8	4.6

Whenever great differences in the coefficients occur, those for the plants grown in hills are always the smaller in amount. It seems, then, that correlation is reduced in amount by allowing more room for the growth and development of plants. Correlation may be considerably modified by the method of growing plants.

The differences produced in the correlation coefficients by differences in the growing conditions may amount to more than any varietal differences that have been observed in the course of this work. By reference to Table 6 it is seen that the range of the differences between the correlation coefficients of different varieties is from .030 to .184, while by reference to Table 9 it is seen that the range of the differences between the correlation coefficients of plants of the same variety grown in drills and in hills is from .002 to .391. Thus the constants obtained by different investigators are comparable only in so far as the conditions of growth are comparable.

Effect of different degrees of crowding on biometrical constants of oats

Are variation and correlation of oats modified by different degrees of crowding in the row? In other words, do oat plants develop in the same way when they are crowded together as thickly as they can stand, as when they are less crowded?

In order to answer these questions, plantings of two pure lines of oats were made in eighteen-foot rows, side by side, on as uniform soil as could be found. One variety was Sixty Day, pure line 62–II–19–2, which had been grown twice from an individual plant and had been propagated for several years by the Department of Plant-Breeding at Cornell University. The other variety was originated by crossing Sixty Day and Probsteier, this having been done some eight or ten years previously. This line, known as 50a1–18–2, was twice selected from the progeny of the cross, and is very uniform. It also has been grown for several years by the Department of Plant-Breeding.

Three different rates of sowings of these lines were made. Lots of 386 kernels and 1000 kernels each were counted out and weighed. Then in each line 40.45 grams was weighed, and the kernels in these weighings were counted. The number of kernels and the weight of the same, for each variety, sown in each eighteen-foot row, are given in the following table, together with the series numbers by which the different sowings are to be known:

TABLE 10. NUMBER AND WEIGHT OF KERNELS SOWN IN EACH EIGHTEEN-FOOT ROW, FOR EACH SERIES USED

Series	Variety	Pure line	Number of kernels sown in 18-foot row	Weight of kernels sown in 18-foot row (grams)
68	Sixty Day	62-II-19-2	386	5.56
102	Sixty Day	62-II-19-2	1,000	15.22
118	Sixty Day	62-II-19-2	2,870	40.45
63	Sixty Day × Probsteier hybrid	50a1-18-2	386	8.11
100	Sixty Day × Probsteier hybrid	50a1-18-2	1,000	20.26
122	Sixty Day × Probsteier hybrid	50a1-18-2	1,925	40.45

These oats were sown on May 12, 1911. The date of pulling and the number of plants harvested in each series from the eighteen-foot rows are given in the following table:

TABLE 11. DATE OF PULLING AND NUMBER OF PLANTS HARVESTED IN EACH ROW, FOR EACH SERIES USED

Series	Date of pulling	Number of plants in 18-foot row
68	July 18, 1911	369
102	July 18, 1911	720
118	July 18, 1911	2,000
63	July 21, 1911	328
100	July 21, 1911	800
122	July 21, 1911	1,500

It is seen that not all the kernels that were planted produced mature plants, yet enough plants matured to show radical differences in the rate of crowding.

The numbers of culms per plant for the 300 plants used for biometrical study of each of these series of oats are given in the following table:

TABLE 12. NUMBER OF CULMS PER PLANT FOR EACH SERIES

Series	Number of culms per plant				Average number of culms per plant
	1	2	3	4	
68	156	102	38	4	1.633
102	275	24	1	....	1.087
118	300	....	....	....	1
63*	147	71	19	1	1.471
100	300	....	....	....	1
122	300	....	....	....	1

*In this series 238 plants were used.

There is very little stooling when 700 plants of these varieties are grown in eighteen-foot rows, and none at all when as many as 1500 are so grown.

The means (M), standard deviations (σ), and coefficients of variability (C) for ten characters of these two lines of oats, sown at these different rates, are given in Tables 13 and 14. In these tables the figures for the least crowded series are given in the left column, those for the most crowded in the right column, and the intermediate in the middle. Line 50a1–18–2 is reported in Table 13, and line 62–II–19–2 in Table 14. These lines will be referred to hereinafter as lines 50 and 62.

TABLE 13. MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY, SERIES 63, 100, 122. DEGREES OF CROWDING. PURE LINE 50a1–18–2

	Average yield of culm per plant, in decigrams		
	63	100	122
M	6.063 ± .108	2.630 ± .065	1.917 ± .047
σ	2.469 ± .076	1.665 ± .046	1.215 ± .033
C	40.72 ± 1.45	63.31 ± 2.34	63.38 ± 2.34
	Average height of plant, in centimeters		
	63	100	122
M	71.785 ± .441	55.850 ± .448	54.983 ± .426
σ	10.100 ± .312	11.526 ± .317	10.950 ± .301
C	14.07 ± .44	20.64 ± .59	19.92 ± .57

TABLE 13. Means, Standard Deviations, and Coefficients of Variability (*continued*)

Average length of head per plant, in centimeters

	63	100	122
M	12.101 ± .097	8.730 ± .084	7.290 ± .079
σ	2.226 ± .069	2.161 ± .059	2.039 ± .056
C	18.40 ± .59	24.75 ± .72	27.97 ± .83

Average weight of kernels per plant, in milligrams

	63	100	122
M	17.882 ± .137	14.943 ± .123	15.527 ± .140
σ	3.134 ± .097	3.154 ± .087	3.603 ± .099
C	17.53 ± .56	21.11 ± .61	23.20 ± .67

Average number of kernels per culm of plant

	63	100	122
M	32.941 ± .516	16.267 ± .357	11.200 ± .255
σ	11.803 ± .365	9.184 ± .253	6.544 ± .180
C	35.83 ± 1.24	56.46 ± 1.99	58.43 ± 2.08

Diameter of straw, in decimillimeters

	63	100	122
M	26.546 ± .191	16.600 ± .195	14.240 ± .166
σ	4.377 ± .135	5.012 ± .138	4.263 ± .117
C	16.49 ± .52	30.19 ± .90	29.94 ± .89

Average weight of straw per culm of plant, in decigrams

	63	100	122
M	7.739 ± .114	4.143 ± .086	2.900 ± .060
σ	2.600 ± .080	2.213 ± .061	1.553 ± .043
C	33.60 ± 1.15	53.42 ± 1.84	53.55 ± 1.85

Average number of spikelets per culm of plant

	63	100	122
M	15.815 ± .270	7.560 ± .164	5.240 ± .111
σ	6.176 ± .191	4.228 ± .116	2.856 ± .079
C	39.05 ± 1.38	55.93 ± 1.96	54.50 ± 1.89

Average number of kernels per spikelet of plant

	63	100	122
M	2.073 ± .011	2.093 ± .012	2.005 ± .012
σ	.253 ± .008	.299 ± .008	.306 ± .008
C	12.20 ± .38	14.29 ± .40	15.26 ± .43

TABLE 13. MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY (*concluded*)

Breaking strength of straw, in grams

	63	100	122
M	226.638 ± 3.704	105.666 ± 1.860	74.734 ± 1.575
σ	84.766 ± 2.619	47.818 ± 1.315	40.500 ± 1.114
C	37.40 ± 1.31	45.25 ± 1.48	54.19 ± 1.88

TABLE 14. MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY, SERIES 68, 102, 118. DEGREES OF CROWDING. PURE LINE 62-II-19-2

Average yield of culm per plant, in decigrams

	68	102	118
M	4.890 ± .081	2.503 ± .066	1.287 ± .039
σ	2.076 ± .057	1.708 ± .047	1.000 ± .027
C	42.45 ± 1.36	68.24 ± 2.61	77.70 ± 3.17

Average height of plant, in centimeters

	68	102	118
M	64.583 ± .420	51.750 ± .507	48.000 ± .429
σ	10.805 ± .297	13.026 ± .358	11.034 ± .303
C	16.73 ± .47	25.17 ± .73	22.99 ± .66

Average length of head per plant, in centimeters

	68	102	118
M	11.123 ± .075	8.283 ± .091	6.257 ± .077
σ	1.933 ± .053	2.327 ± .064	1.976 ± .054
C	17.38 ± .49	28.09 ± .83	31.58 ± .95

Average weight of kernels per plant, in milligrams

	68	102	118
M	13.197 ± .074	10.917 ± .103	10.490 ± .101
σ	1.895 ± .052	2.655 ± .073	2.592 ± .071
C	14.36 ± .40	24.32 ± .71	24.71 ± .72

Average number of kernels per culm of plant

	68	102	118
M	36.013 ± .528	20.787 ± .507	10.453 ± .298
σ	13.586 ± .374	13.026 ± .358	7.660 ± .211
C	37.73 ± 1.18	62.66 ± 2.30	73.28 ± 2.90

Diameter of straw, in decimillimeters

	68	102	118
M	23.907 ± .181	17.760 ± .210	12.520 ± .171
σ	4.648 ± .128	5.405 ± .149	4.386 ± .121
C	19.44 ± .55	30.43 ± .91	35.03 ± 1.08

TABLE 14. Means, Standard Deviations, and Coefficients of Variability (*concluded*)

	Average weight of straw per culm of plant, in decigrams		
	68	102	118
M	5.963 ± .084	3.767 ± .087	2.103 ± .052
σ	2.167 ± .060	2.243 ± .062	1.341 ± .037
C	36.34 ± 1.12	59.54 ± 2.14	63.77 ± 2.36
	Average number of spikelets per culm of plant		
	68	102	118
M	17.027 ± .267	9.227 ± .215	4.707 ± .118
σ	6.857 ± .189	5.523 ± .152	3.023 ± .083
C	40.27 ± 1.27	59.86 ± 2.16	64.22 ± 2.39
	Average number of kernels per spikelet of plant		
	68	102	118
M	2.013 ± .012	2.135 ± .015	2.046 ± .012
σ	.311 ± .009	.379 ± .010	.312 ± .009
C	15.45 ± .43	17.75 ± .50	15.25 ± .43
	Breaking strength of straw, in grams		
	68	102	118
M	209.346 ± 3.100	84.534 ± 1.985	58.400 ± 1.475
σ	79.696 ± 2.192	51.036 ± 1.403	37.930 ± 1.043
C	38.07 ± 1.19	60.37 ± 2.18	64.95 ± 2.43

Considering first the average yield of culm per plant in decigrams, it is seen in Table 13 that the mean for series 63 is 6.063 ± .108, for series 100 it is 2.630 ± .065, and for series 122 it is 1.917 ± .047. It is evident that the yield per culm averages higher for plants grown in the less crowded conditions. Without repeating here the means for the other line, the same results are seen to hold in this. As to variability, considering the standard deviation to be in such cases the best index, it is clear that the variability in yield of culm per plant is greatest in the plants grown under the least crowded conditions. The standard deviations for series 63, 100, and 122, are, respectively, 2.469 ± .076, 1.665 ± .046, and 1.215 ± .033. The other line exhibits about the same decrease in standard deviations from the least to the most crowded condition.

It will probably be well to take up separately the remaining characters considered, but without repeating in the text the constants recorded in the tables unless some unusual circumstance exists. Reference can be made to the tables for the constants on which conclusions are based.

There is a decrease in average height of plant in centimeters from the least to the most crowded conditions; the extreme differences being 16.802 centimeters in one line, and 16.583 centimeters in the other. As to variability, however, the plants growing under the least crowded conditions show the least variation, while those in intermediate conditions of crowding show the greatest variation. This is true for both lines.

The means for average length of head per plant in centimeters decrease as crowding increases. In line 50 the variability decreases in like manner, but the differences are slight. In line 62 the variability decreases in the same order as does that for height.

For average weight of kernels per plant in milligrams, the means decrease in regular order from the least to the most crowded condition in line 62, but the extreme difference is but 2.707 ± .125 milligrams. In line 50 the greatest weight occurs in series 63, the second greatest in series 122, and the least in series 100, the intermediate condition of crowding. The extreme difference here is 2.939 ± .184 milligrams. Variability is least in both lines in the least crowded conditions, but the differences are not great.

The average number of kernels per culm of plant decreases markedly as crowding increases; the extreme differences being 21.741 ± .576 for line 50, and 25.560 ± .606 for line 62. The variability likewise decreases as the plants have less room.

The average number of spikelets per culm of plant should be considered here, since it is so closely related to the number of kernels. The same conditions exist for the two characters. The extreme differences in the means for number of spikelets are 10.575 ± .292 for line 50, and 12.320 ± .292 for line 62, and the variability is also less as more crowded conditions prevail.

The average number of kernels per spikelet of plant should now be considered. The differences here are small, but in both lines the mean is highest when conditions of crowding are intermediate; it is lowest when crowding is greatest in line 50, and at the other extreme in line 62. Variability increases directly with crowding in line 50, but in line 62 the plants in the intermediate condition of crowding have the greatest variability, while the two extremes are practically the same.

Three lots of data have been taken on the straw: the diameter, in decimillimeters; the average weight of straw per culm of plant, in decigrams; and the breaking strength, in grams. For plants with more than one culm the diameter and the breaking strength were determined for the principal culm, according to methods described previously. The means for all three of these characters are seen to decrease markedly as the degree of crowding increases. The variability decreases for weight in line 50, and for breaking strength in both lines, with decrease in amount of room allowed to the plants. In line 62, however, the greatest variability in weight of straw occurs in series 102, where crowding is intermediate, but the least variability is again where the greatest degree of crowding exists. In diameter of straw the variability is greatest in both lines where the intermediate degree of crowding exists, but is least where the crowding is greatest.

Oat plants grown in very crowded positions produce but one culm to a plant, but as more room is given more than one culm are produced by many plants. Tillering ceases in these varieties when the rate of stand is somewhere about forty to forty-five plants to a foot. The development of the plants in less crowded conditions is greater than in more crowded conditions, as is evidenced by the means for yield, height, length of head, weight, diameter, and breaking strength of straw, number of spikelets per culm, and number of kernels per culm. The kernels also average heaviest when crowding is least, but there is not much difference in weight for conditions of crowding such as exist in series 100, 122, 102, and 118. The numbers of kernels produced by the spikelets do not differ greatly for the different rates of seeding; the number produced on the plants growing under medium conditions of crowding, however, is slightly the largest.

Variability, as indicated by the standard deviation, decreases in both lines with decrease in the mean in yield, number of kernels per culm, number of spikelets per culm, and breaking strength of straw. In both lines the variability for height of plant and diameter of straw is greatest for the medium thick stand. For height the least variability occurs where the most room per plant is allowed, but for diameter of straw the least variability occurs when the crowding is greatest. In line 50, variability increases with crowding for weight of kernels per plant and for number of kernels per spikelet, and decreases for weight of straw and for length of head. In line 62, variability is greatest in the medium thick stand for length of head, weight of kernels, number of kernels per spikelet, and weight of straw, and least in the least crowded condition for the first three of these characters; for weight of straw it is least in the most crowded condition.

Correlations

The correlations that exist in the two lines under consideration sown at the different rates are given in Tables 15 and 16. Thirteen correlation coefficients for each series have been determined, and these are given

TABLE 15. CORRELATIONS, SERIES 63, 100, 122. DEGREES OF CROWDING. PURE LINE 50a1-18-2

Average yield of culm per plant, in decigrams — Average height of plant, in centimeters	63..... r = .811 ± .015	100.... r = .881 ± .009	122.... r = .865 ± .010
Average yield of culm per plant, in decigrams — Average length of head per plant, in centimeters	63..... r = .774 ± .018	100.... r = .847 ± .011	122.... r = .849 ± .011
Average yield of culm per plant, in decigrams — Average number of kernels per culm of plant	63..... r = .906 ± .008	100.... r = .930 ± .005	122.... r = .908 ± .007
Average yield of culm per plant, in decigrams — Average number of spikelets per culm of plant	63..... r = .845 ± .012	100.... r = .885 ± .008	122.... r = .851 ± .011
Average yield of culm per plant, in decigrams — Average weight of kernels per plant, in milligrams	63..... r = .492 ± .033	100.... r = .585 ± .026	122.... r = .652 ± .022
Average yield of culm per plant, in decigrams — Average number of kernels per spikelet of plant	63... r = —.025 ± .044	100.... r = .404 ± .033	122.... r = .489 ± .030
Average yield of culm per plant, in decigrams — Diameter of straw, in decimillimeters	63..... r = .765 ± .018	100.... r = .882 ± .009	122.... r = .866 ± .010
Average yield of culm per plant, in decigrams — Breaking strength of straw, in grams	63..... r = .673 ± .024	100.... r = .896 ± .008	122.... r = .870 ± .009
Average yield of culm per plant, in decigrams — Average weight of straw per culm of plant, in decigrams	63..... r = .919 ± .007	100.... r = .941 ± .004	122.... r = .901 ± .007
Diameter of straw, in decimillimeters — Breaking strength of straw, in grams	63..... r = .820 ± .014	100.... r = .852 ± .011	122.... r = .913 ± .006
Average weight of kernels per plant, in milligrams — Average number of kernels per culm of plant	63..... r = .133 ± .043	100.... r = .363 ± .034	122.... r = .466 ± .030

TABLE 15. CORRELATIONS, SERIES 63, 100, 122. DEGREES OF CROWDING (*concluded*)

Average weight of kernels per plant, in milligrams — Average height of plant, in centimeters

63..... r = .380 ± .037   100.... r = .651 ± .022   122.... r = .718 ± .019

Average weight of kernels per plant, in milligrams — Average length of head per plant, in centimeters

63..... r = .081 ± .043   100.... r = .408 ± .032   122.... r = .535 ± .028

TABLE 16. CORRELATIONS, SERIES 68, 102, 118. DEGREES OF CROWDING. PURE LINE 62–II–19–2

Average yield of culm per plant, in decigrams — Average height of plant, in centimeters

68..... r = .836 ± .012   102.... r = .863 ± .010   118.... r = .801 ± .014

Average yield of culm per plant, in decigrams — Average length of head per plant, in centimeters

68..... r = .811 ± .013   102.... r = .835 ± .012   118.... r = .800 ± .014

Average yield of culm per plant, in decigrams — Average number of kernels per culm of plant

68..... r = .942 ± .004   102.... r = .954 ± .003   118.... r = .928 ± .005

Average yield of culm per plant, in decigrams — Average number of spikelets per culm of plant

68..... r = .900 ± .007   102.... r ± .913 ± .006   118.... r = .892 ± .008

Average yield of culm per plant, in decigrams — Average weight of kernels per plant, in milligrams

68..... r = .525 ± .028   102.... r = .583 ± .026   118.... r = .568 ± .026

Average yield of culm per plant, in decigrams — Average number of kernels per spikelet of plant

68..... r = .060 ± .039   102.... r = .474 ± .030   118.... r = .621 ± .024

Average yield of culm per plant, in decigrams — Diameter of straw, in decimillimeters

68..... r = .785 ± .015   102.... r = .878 ± .009   118.... r = .871 ± .009

Average yield of culm per plant, in decigrams — Breaking strength of straw, in grams

68..... r = .701 ± .020   102.... r ± .863 ± .010   118.... r = .883 ± .009

Average yield of culm per plant, in decigrams — Average weight of straw per culm of plant, in decigrams

68..... r = .884 ± .008   102.... r = .944 ± .004   118.... r = .888 ± .008

Diameter of straw, in decimillimeters — Breaking strength of straw, in grams

68..... r = .871 ± .009   102.... r = .960 ± .003   118.... r = .899 ± .007

TABLE 16. CORRELATIONS, SERIES 68, 102, 118. DEGREES OF CROWDING (*concluded*)

Average weight of kernels per plant, in milligrams — Average number of kernels per culm of plant		
68 . . . . r = .288 ± .036	102 . . . . r = .471 ± .030	118 . . . . r = .487 ± .030
Average weight of kernels per plant, in milligrams — Average height of plant, in centimeters		
68 . . . . r = .450 ± .031	102 . . . . r = .733 ± .018	118 . . . . r = .714 ± .019
Average weight of kernels per plant, in milligrams — Average length of head per plant, in centimeters		
68 . . . . r = .265 ± .036	102 . . . . r = .535 ± .028	118 . . . . r = .606 ± .025

immediately beneath the characters correlated. Those for the least crowded condition are in the first column at the left, those for the most crowded condition are in the column at the right.

The average yield of culm per plant has been correlated with nine different characters. Taking up for consideration the correlation between this character and average height of plant (Figs. 121 to 126), it is seen that the highest correlation for line 50 is in series 100, where it is .881 ± .009; and for line 62 in series 102, where it is .863 ± .010. These are

Series 63

	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	Totals
1– 2	1	1	5	3	3							13
2– 3			3	4	2	2						11
3– 4	1		2	7	10	6	2	1				29
4– 5				2	4	13	5	2				26
5– 6			1	2	3	14	14	5	1			40
6– 7					3	4	17	12	8			44
7– 8						1	5	10	3	5		24
8– 9						1	2	6	6	3		18
9–10							1	3	7	4	2	17
10–11							2	1	2	3	1	9
11–12										3	2	5
12–13								1		1		2
Totals	2	1	11	18	25	41	48	41	27	19	5	238

FIG. 121.— *Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative*
r = .811 ± .015

Series 100

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Totals
0–1	1	10	17	15	3								46
1–2		2	5	6	29	30	5						77
2–3					1	17	29	28	1				76
3–4					1	1	4	20	19				45
4–5							1	4	13	6			24
5–6								2	3	11	2		18
6–7									2	5	3	1	11
7–8											1		1
8–9											1	1	2
Totals	1	12	22	21	34	48	39	54	38	22	7	2	300

FIG. 122.—*Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative*

r = .881 ± .009

.070 ± .017 and .062 ± .017 higher than the lowest coefficients in their respective lines. Each of these differences is about four times its probable error and they are not highly significant. The correlations are highest in both lines for plants grown in intermediate conditions of crowding

Series 122

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	Totals
0–1	1	10	25	19	19	4						78
1–2	1				20	40	28	6	1			96
2–3						3	22	29	16	2		72
3–4								11	22	4		37
4–5									5	5	2	12
5–6										2	2	4
6–7											1	1
Totals	2	10	25	19	39	47	50	46	44	13	5	300

FIG. 123.—*Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative*

r = .865 ± .010

Series 68

	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	Totals
0– 1	2	2	3											7
1– 2		2	3	7	4	4	1							21
2– 3			2	4	8	10	1	1						26
3– 4				2	8	24	13	5	1					53
4– 5					1	11	18	17	6	2				55
5– 6						2	12	12	10	4	1	1		42
6– 7							6	16	10	8	1			41
7– 8								7	6	14	10			37
8– 9								2	2	3	2	3		12
9–10								1	1		2	1	1	6
Totals	2	4	8	13	21	51	51	61	36	31	16	5	1	300

FIG. 124.— *Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative*
r = .836 ± .012

and lowest, in one case for those in the least crowded condition, and in the other case for those in the most crowded condition. This would give a slight indication that yield increases in its correlation with height as crowding increases up to a certain point, beyond which there is a gradual decrease in correlation.

Series 102

	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	Totals
0–1	1	2	6	13	12	19	10								63
1–2						3	19	27	23	6					78
2–3								6	30	17	6				59
3–4									4	11	18	6	1		40
4–5									1	4	9	13	5		32
5–6											2	7	2	2	13
6–7											1	5	6	2	14
7–8															0
8–9													1		1
Totals	1	2	6	13	12	22	29	33	58	38	36	31	15	4	300

FIG. 125.— *Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative*
r = .863 ± .010

Series 118

	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	Totals
0–1	4	11	23	34	46	26	9						153
1–2				1	3	21	29	27	4	1			86
2–3					1	1	6	12	15	5			40
3–4								1	7	5	2		15
4–5									1	2	1	1	5
5–6											1		1
Totals	4	11	23	35	50	48	44	40	27	13	4	1	300

FIG. 126.—*Correlation between average yield of culm per plant, in decigrams, subject; and average height of plant, in centimeters, relative*

r = .801 ± .014

When average yield of culm per plant is correlated with average length of head per plant (Figs. 127 to 132), the coefficients are not graduated so uniformly in the two lines. The greatest correlation in line 50 occurs in series 122, being here .849 ± .011. This is for the most crowded

Series 63

	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	Totals
1– 2	3	5	2	2	1							13
2– 3	1	1	2	4		2	1					11
3– 4	1		7	9	4	7	1					29
4– 5				1	5	10	8	2				26
5– 6			2	1	6	13	7	8	2	1		40
6– 7			1	3	4	4	14	11	6	1		44
7– 8					1	2	2	10	8	1		24
8– 9						1	6	4	2	5		18
9–10							2	8	2	4	1	17
10–11								2	3	4		9
11–12							1		1	3		5
12–13											2	2
Totals	5	6	14	20	21	39	42	45	24	19	3	238

FIG. 127.—*Correlation between average yield of culm per plant, in decigrams, subject; and average length of head per plant, in centimeters, relative*

r = .774 ± .018

Series 100

	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	Totals
0–1	2	11	15	11	7								46
1–2			3	21	23	21	9						77
2–3				2	10	35	21	8					76
3–4						9	17	11	8				45
4–5						1	5	4	8	3	2	1	24
5–6							2	3	6	7			18
6–7								1	3	3	2	2	11
7–8										1			1
8–9									1		1		2
Totals	2	11	18	34	40	66	54	27	26	14	5	3	300

FIG. 128.— *Correlation between average yield of culm per plant, in decigrams, subject; and average length of head per plant, in centimeters, relative*

r = .847 ± .011

condition, and the coefficients become lower with less crowding, the lowest coefficient being .774 ± .018, or .075 ± .021 less than the highest. This difference is more than three times its probable error, and probably is not significant. In line 62 the correlation is greatest for the medium

Series 122

	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	Totals
0–1	2	15	27	27	7						78
1–2				15	31	38	9	3			96
2–3					3	20	34	10	4	1	72
3–4						3	9	13	8	4	37
4–5								3	4	5	12
5–6									3	1	4
6–7								1			1
Totals	2	15	27	42	41	61	52	30	19	11	300

FIG. 129.— *Correlation between average yield of culm per plant, in decigrams, subject; and average length of head per plant, in centimeters, relative*

r = .849 ± .011

Series 68

	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	Totals
0–1	4	2	1									7
1–2		3	7	4	7							21
2–3			4	4	12	5	1					26
3–4			1	6	16	16	10	2			2	53
4–5				1	8	15	24	5	2			55
5–6					1	7	18	14	1	1		42
6–7						2	10	18	10	1		41
7–8							7	12	15	3		37
8–9							1	4	4	2	1	12
9–10								1	2	3		6
Totals	4	5	13	15	44	45	71	56	34	10	3	300

FIG. 130.— *Correlation between average yield of culm per plant, in decigrams, subject; and average length of head per plant, in centimeters, relative*

r = .811 ± .013

condition of crowding and least for the most crowded condition, but the extreme difference is only .035 ± .018. Only slight significance can be attached to the differences in correlation in these two lines between yield and length of head, therefore definite conclusions cannot be drawn.

Series 102

	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	Totals
0–1	5	6	21	15	12	4							63
1–2				5	26	26	15	6					78
2–3					1	4	23	24	6			1	59
3–4							5	12	17	6			40
4–5							2	10	10	8	2		32
5–6								2	3	7	1		13
6–7									4	7	3		14
7–8													0
8–9											1		1
Totals	5	6	21	20	39	34	45	54	40	28	7	1	300

FIG. 131.—*Correlation between average yield of culm per plant, in decigrams, subject; and average length of head per plant, in centimeters, relative*

r = .835 ± .012

Series 118

	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	Totals
0–1	2	14	31	27	51	22	6					153
1–2					9	33	38	6				86
2–3						1	10	18	9	2		40
3–4							1	4	6	4		15
4–5									5			5
5–6											1	1
Totals	2	14	31	27	60	56	55	28	20	6	1	300

FIG. 132.— *Correlation between average yield of culm per plant, in decigrams, subject; and average length of head per plant, in centimeters, relative*

r = .800 ± .014

When the correlation between average yield of culm per plant and average number of kernels per culm of plant (Figs. 133 to 138) is considered, the differences in the correlations are so small in each line as to be of little or no significance. There is a slightly greater correlation in each line,

Series 63

	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	68–72	Totals
1– 2	7	5	1														13
2– 3	1	6	2	1	1												11
3– 4		3	8	12	5	1											29
4– 5				4	10	8	4										26
5– 6				4	10	9	13	4									40
6– 7					5	9	10	11	6	2	1						44
7– 8					1	1	3	5	11	2	1						24
8– 9							2	1	4	6	3	2					18
9–10								2	3	2	8	1		1			17
10–11										4	2	3					9
11–12										1	1	2				1	5
12–13													2				2
Totals	8	14	11	21	32	28	32	23	24	17	16	8	2	1	0	1	238

FIG. 133.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative*

r = .906 ± .008

Series 100

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	Totals
0–1	5	37	4									46
1–2		17	45	15								77
2–3			9	39	23	3	2					76
3–4				5	12	19	8	1				45
4–5				1		5	6	11	1			24
5–6							3	5	5	5		18
6–7							1	2	3	3	2	11
7–8										1		1
8–9										1	1	2
Totals	5	54	58	60	35	27	20	19	9	10	3	300

Fig. 134.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative*

r = .930 ± .005

however, for the medium condition of crowding. The smallest coefficient is .906 ± .008, for series 63. The extreme difference of the results in line 50 is .024 ± .009, which is less than three times its probable error. In

Series 122

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	Totals
0–1	34	44							78
1–2		35	50	10	1				96
2–3			15	42	13	2			72
3–4				8	14	12	3		37
4–5						3	7	2	12
5–6							4		4
6–7							1		1
Totals	34	79	65	60	28	17	15	2	300

Fig. 135.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative*

r = .908 ± .007

Series 68

	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	Totals
0–1	5	2															7
1–2		6	12	2	1												21
2–3			2	8	10	5	1										26
3–4				3	8	19	18	5									53
4–5						4	13	15	16	4	3						55
5–6								6	14	14	6	2					42
6–7									1	11	16	8	5				41
7–8										3	8	8	8	5	5		37
8–9											1		6	3	2		12
9–10													1	1	3	1	6
Totals	5	8	14	13	19	28	32	26	31	32	34	18	20	9	10	1	300

FIG. 136.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative*
r = .942 ± .004

line 62 it is .026 ± .006, or about four times its probable error. Degrees of crowding, then, have little effect on the correlation between yield and number of kernels per culm.

Series 102

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	Totals
0–1	17	30	16													63
1–2		2	29	33	11	2	1									78
2–3				5	19	25	10									59
3–4						3	12	14	8	3						40
4–5						1	1	6	9	10	5					32
5–6										1	5	5	2			13
6–7										1	4	3	2	3	1	14
7–8																0
8–9															1	1
Totals	17	32	45	38	30	31	24	20	17	15	14	8	4	3	2	300

FIG. 137.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative*
r = .954 ± .003

Series 118

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	Totals
0–1	52	86	15								153
1–2		4	53	26	3						86
2–3			1	5	16	13	4	1			40
3–4					1	2	4	7	1		15
4–5								3	2		5
5–6										1	1
Totals	52	90	69	31	20	15	8	11	3	1	300

FIG. 138.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per culm of plant, relative*

r = .928 ± .005

The correlations between average yield of culm per plant and average number of spikelets per culm of plant (Figs. 139 to 144) are again so nearly the same in amount that no significance may be placed on the difference. In line 50 the extreme difference is .040 ± .014; in line 62 it is .021 ± .010.

Series 63

	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	Totals
1– 2	1	7	4	1														13
2– 3	1	2	4	3			1											11
3– 4			8	8	8	3	2											29
4– 5				3	1	7	11	4										26
5– 6			1		7	6	9	10	6	1								40
6– 7				1	7	3	5	12	8	4	3		1					44
7– 8					1	1	3	3	6	4	5	1						24
8– 9						1	1	2	1	4	3	3	3					18
9–10							2	1	1	4	3	4	1		1			17
10–11										1	3	2	3					9
11–12											2	1	1				1	5
12–13														1		1		2
Totals	2	9	17	16	24	21	34	32	22	18	19	11	9	1	1	1	1	238

FIG. 139.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative*

r = .845 ± .012

Series 100

	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	Totals
0–1	5	34	7										46
1–2		20	42	15									77
2–3			25	30	15	4	2						76
3–4			3	13	12	11	6						45
4–5				2	2	5	6	6	3				24
5–6					1		6	7	3	1			18
6–7							4	2	1	4			11
7–8									1				1
8–9								1				1	2
Totals	5	54	77	60	30	20	24	16	8	5	0	1	300

FIG. 140.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative*

r = .885 ± .008

The difference in each case amounts to less than three times its probable error. Degrees of crowding, then, have little effect on the correlation between yield and number of spikelets.

Series 122

	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	Totals
0–1	29	48	1						78
1–2		34	51	11					96
2–3		1	34	26	11				72
3–4			5	11	11	7	3		37
4–5					2	7	2	1	12
5–6					2	1	1		4
6–7						1			1
Totals	29	83	91	48	26	16	6	1	300

FIG. 141.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative*

r = .851 ± .011

Series 68

	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	Totals
0– 1	4	3															7
1– 2		9	8	4													21
2– 3			7	6	7	4	2										26
3– 4			5	5	14	12	11	6									53
4– 5				2	1	9	20	7	11	2	3						55
5– 6						2	2	9	11	12	5				1		42
6– 7							1	2	2	14	11	5	5	1			41
7– 8								1	3	6	6	11	2	7	1		37
8– 9										1	1	1	4	2	3		12
9–10										1		1			3	1	6
Totals	4	12	20	17	22	27	36	25	27	36	26	18	11	10	8	1	300

FIG. 142.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative*

r = .900 ± .007

Series 102

	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	Totals
0–1	14	32	16	1											63
1–2		6	36	24	9	2	1								78
2–3			4	11	26	12	5	1							59
3–4					7	2	14	14	3						40
4–5					2	4	5	9	9	2	1				32
5–6							1	1	4	6		1			13
6–7								2	2	4	2		2	2	14
7–8															0
8–9													1		1
Totals	14	38	56	36	44	20	26	27	18	12	3	1	3	2	300

FIG. 143.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative*

r = .913 ± .006

Series 118

	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	Totals
0–1	50	89	14							153
1–2		7	57	21	1					86
2–3			2	17	16	5				40
3–4			1	2	4	3	4	1		15
4–5						2	2	1		5
5–6									1	1
Totals	50	96	74	40	21	10	6	2	1	300

FIG. 144.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of spikelets per culm of plant, relative*

r = .892 ± .008

In the correlation between average yield of culm per plant and average weight of kernels per plant (Figs. 145 to 150), there is in line 50 a graduated increase with increased crowding. For series 63 the coefficient is .492 ± .033, while for series 122 it is .652 ± .022 — an extreme difference of .160 ± .040, which, however, amounts to but four times its probable

Series 63

	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	24–25	25–26	26–27	Totals
1– 2	1		2	1	2	3	2	2											13
2– 3	1			2		2	1	1		1		1	1	1					11
3– 4				1	3	5	6	4	3	2	2	2			1				29
4– 5					2	9	4	4	1	2	2	1	1						26
5– 6						5	6	6	8	2	6		1	3	2	1			40
6– 7				1		2	5	7	6	8	1	3	4	2	2	1	1	1	44
7– 8						1	1	5	5	2	4	2	1	1	1			1	24
8– 9							3	2	2	3	3	2	1			2			18
9–10						1			3	3	3	2	1	3			1		17
10–11										1	2	2	3	1					9
11–12								1				2		1	1				5
12–13												1	1						2
Totals	2	0	2	5	7	28	28	32	28	24	23	18	14	12	7	4	2	2	238

FIG. 145.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative*

r = .492 ± .033

Series 100

	4–5	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	25–26	27–28	Totals
0–1	1	2	1	3	10	10	11	7	1													46
1–2						2	8	8	18	16	9	7	1	5	3							77
2–3							3	7	6	16	12	7	10	7	4	1	2		1			76
3–4								2	2	12	10	4	5	4	5	1						45
4–5									1	5	9	3		2	2	1					1	24
5–6									1	3	4	2	4	1	1	2						18
6–7										2	1	1	2	1	2			1		1		11
7–8														1								1
8–9														1				1				2
Totals	1	2	1	3	10	12	22	24	29	54	45	24	22	22	17	5	2	2	1	1	1	300

FIG. 146.*— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative*

r = .585 ± .026

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* See footnote to Fig. 73, page 93.

Series 122

	6– 7	7– 8	8– 9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	20–21	21–22	22–23	23–24	30–31	Totals
0–1	1	5	8	9	11	17	7	7	4	4	3	1		1						78
1–2						5	10	12	12	12	12	15	15	1	1			1		96
2–3							2	2	4	13	10	12	10	11	3	3	1		1	72
3–4							1	2	2	1	6	6	7	4	2	4	1	1		37
4–5										2	1	3	3	2			1			12
5–6														1	3					4
6–7																		1		1
Totals	1	5	8	9	11	22	20	23	22	32	32	37	35	20	9	7	3	3	1	300

FIG. 147.*— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative*

r = .652 ± .022

error. In line 62 the greatest correlation is found where crowding is medium, and the least correlation where crowding is least. For series 102 the coefficient is .583 ± .026, and for series 68 it is .525 ± .028; the extreme difference being .058 ± .038, which is only about half as much

Series 68

	8– 9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	Totals
0– 1	3	2	1	1									7
1– 2	3	2	2	7	3	2	2						21
2– 3		4	5	7	3	4	1	2					26
3– 4			8	6	16	6	7	5	4		1		53
4– 5		1	6	9	13	14	6	1	4	1			55
5– 6			1	2	10	14	10	5					42
6– 7				3	7	17	7	6	1				41
7– 8				3	5	8	8	8	2	3			37
8– 9						1	6	4		1			12
9–10							3	2	1				6
Totals	6	9	23	38	57	66	50	33	12	5	1	0	300

FIG. 148.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative*

r = .525 ± .028

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* See footnote to Fig. 73, page 93.

Series 102

	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	Totals
0–1	8	6	8	5	5	8	11	9	1	2						63
1–2				1	3	5	15	11	18	11	10	2	2			78
2–3						1	7	13	17	11	3	5	2			59
3–4						1	1	14	8	10	4		1	1		40
4–5								4	8	3	11	4	1		1	32
5–6								1	4	4	3		1			13
6–7									1	5	2	4	2			14
7–8																0
8–9											1					1
Totals	8	6	8	6	8	15	34	52	57	46	34	15	9	1	1	300

Fig. 149.—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative*
$r = .583 \pm .026$

more than its probable error. There is, then, only a slight tendency shown for the correlation between yield and average weight of kernels to increase as crowding increases.

Series 118

	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	20–21	Totals
0–1	1	3	8	19	25	26	27	20	16	6	1	1				153
1–2						2	8	14	17	20	14	7	3		1	86
2–3						2		7	10	8	8	3	1	1		40
3–4								3	5	4		1		2		15
4–5										2	1	1	1			5
5–6											1					1
Totals	1	3	8	19	25	30	35	44	48	40	25	13	5	3	1	300

Fig. 150.*—*Correlation between average yield of culm per plant, in decigrams, subject; and average weight of kernels per plant, in milligrams, relative*
$r = .568 \pm .026$

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* See footnote to Fig. 73, page 93.

Very different results from those that have heretofore been encountered in these lines are found when average yield of culm per plant is correlated with average number of kernels per spikelet of plant (Figs. 151 to 156). In both lines there is a graduated increase from the condition of least crowding to that of most crowding. In line 50 the extremes are found in series 63 and series 122, the respective coefficients being — .025 ± .044 and .489 ± .030, an extreme difference of .514 ± .053, which is nearly ten times its probable error. In line 62 the extremes are in series 68 and series 118, being .060 ± .039 and .621 ± .024, respectively — an extreme difference of .561 ± .046, which is about twelve times its probable error. There is, then, a decided and significant increase in correlation between yield and number of kernels per spikelet as crowding increases. The means and the standard deviations for the former character decreased with crowding; for the latter character there was not much difference produced by crowding.

Series 63

	1.55–1.65	1.65–1.75	1.75–1.85	1.85–1.95	1.95–2.05	2.05–2.15	2.15–2.25	2.25–2.35	2.35–2.45	2.45–2.55	2.55–2.65	2.65–2.75	2.75–2.85	2.85–2.95	2.95–3.05	Totals
1– 2			1	2	6	1	1				1	1				13
2– 3	1		1	3		1		2			2				1	11
3– 4		2	4	4	4	2	5	4	1		3					29
4– 5		1	3	9	3	6	2		1	1						26
5– 6		2	5	15	4	6	4		1	2		1				40
6– 7		1	2	12	10	3	5	2	1	2	3	2	1			44
7– 8			2	8	7		3	1	1		1	1				24
8– 9		1	1	1	6	4	2	1		1			1			18
9–10				5	4	2		3	1	1		1				17
10–11			1	3	3		1			1						9
11–12				1	3			1								5
12–13		1			1											2
Totals	1	8	20	63	51	25	23	14	6	8	10	6	2	0	1	238

FIG. 151.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per spikelet of plant, relative*
r = — .025 ± .044

Series 100

	1.35–1.45	1.45–1.55	1.55–1.65	1.65–1.75	1.75–1.85	1.85–1.95	1.95–2.05	2.05–2.15	2.15–2.25	2.25–2.35	2.35–2.45	2.45–2.55	2.55–2.65	2.65–2.75	2.75–2.85	2.85–2.95	2.95–3.05	3.05–3.15	3.15–3.25	Totals
0–1	1	2	2	13	2		26													46
1–2		1	2	7	11	3	40	2	10			1								77
2–3			2	1	5	9	17	9	3	11	5	5	4	3	1		1			76
3–4					2	4	7	9	1	4	4	1	2	5	1	3		1	1	45
4–5					2	3	4	4	4	2	1		3	1						24
5–6					3		1		5	2	4	2		1						18
6–7						2	3	1		2		2	1							11
7–8									1											1
8–9						1							1							2
Totals	1	3	6	21	25	22	98	25	24	21	14	11	11	10	2	3	1	1	1	300

FIG. 152.—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per spikelet of plant, relative*

r = .404 ± .033

Series 122

	0.95–1.05	1.05–1.15	1.15–1.25	1.25–1.35	1.35–1.45	1.45–1.55	1.55–1.65	1.65–1.75	1.75–1.85	1.85–1.95	1.95–2.05	2.05–2.15	2.15–2.25	2.25–2.35	2.35–2.45	2.45–2.55	2.55–2.65	2.65–2.75	2.75–2.85	2.85–2.95	2.95–3.05	Totals
0–1	1			5		11	1	15			45											78
1–2					2		3	10	17	1	54		5	1	1			1			1	96
2–3							1	3	1	8	26	5	11	7		1	3	1	3		2	72
3–4								1	3	5	8	5	1	1	4	1	3	2	2	1		37
4–5										1	1	4	2	1	1		1	1				12
5–6									1				1				1				1	4
6–7													1									1
Totals	1	0	0	5	2	11	5	29	22	15	134	14	21	10	6	2	8	5	5	1	4	300

FIG. 153.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per spikelet of plant, relative*

r = .489 ± .030

Series 68	1.35–1.45	1.45–1.55	1.55–1.65	1.65–1.75	1.75–1.85	1.85–1.95	1.95–2.05	2.05–2.15	2.15–2.25	2.25–2.35	2.35–2.45	2.45–2.55	2.55–2.65	2.65–2.75	2.75–2.85	2.85–2.95	2.95–3.05	Totals
0– 1			2				4							1				7
1– 2				1	2	1	9		2	2	2		1	1				21
2– 3		1		1	4	5	7	2	1	1		1	1	1			1	26
3– 4				1	2	13	8	9	2	4	2	2	2	3	1		4	53
4– 5					4	10	11	7	4	3	4	5	1	2	2	1	1	55
5– 6	1				1	5	16	9	2	3		1	3	1				42
6– 7				1	2	5	19	3	4	2	1	2	1	1				41
7– 8					1	3	10	10	5		3	2	2		1			37
8– 9					1	1	7	2	1									12
9–10						2		2	1						1			6
Totals	1	1	2	4	17	45	91	44	22	15	12	13	11	10	5	1	6	300

FIG. 154.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per spikelet of plant, relative*

r = .060 ± .039

Series 102

	0.95–1.05	1.05–1.15	1.15–1.25	1.25–1.35	1.35–1.45	1.45–1.55	1.55–1.65	1.65–1.75	1.75–1.85	1.85–1.95	1.95–2.05	2.05–2.15	2.15–2.25	2.25–2.35	2.35–2.45	2.45–2.55	2.55–2.65	2.65–2.75	2.75–2.85	2.85–2.95	2.95–3.05	Totals
0–1	4		1	1		9	1	10	7		29							1				63
1–2						1	2	6	9	8	24	3	4	3	4	2	4	2	3		3	78
2–3							1	2	3	2	9	8	9	6	6	5	1	3	1		3	59
3–4									1	6	4	5	5	7	4	2	2		2	1	1	40
4–5										2	3	5	3	3	3	3	4	4	1		1	32
5–6											1	1	1	2	2	2	1	1	1	1		13
6–7										1	3	2	1	1	1		1	1	1	1	1	14
7–8																						0
8–9														1								1
Totals	4	0	1	1	0	10	4	18	20	19	73	24	23	23	20	14	13	12	9	3	9	300

FIG. 155.— *Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per spikelet of plant, relative*

r = .474 ± .030

Series 118

	0.95–1.05	1.05–1.15	1.15–1.25	1.25–1.35	1.35–1.45	1.45–1.55	1.55–1.65	1.65–1.75	1.75–1.85	1.85–1.95	1.95–2.05	2.05–2.15	2.15–2.25	2.25–2.35	2.35–2.45	2.45–2.55	2.55–2.65	2.65–2.75	2.75–2.85	2.85–2.95	2.95–3.05	Totals
0–1	2			2		11	1	22	6		107		1								1	153
1–2							2	2	7	5	49	4	3	6			2	2	3		1	86
2–3										1	4	9	4	6	6	3	4			2	1	40
3–4												1	2		1	2	4	1	1	1	2	15
4–5													1		1	1	1			1		5
5–6															1							1
Totals	2	0	0	2	0	11	3	24	13	6	160	14	11	12	9	6	11	3	4	4	5	300

FIG. 156.—*Correlation between average yield of culm per plant, in decigrams, subject; and average number of kernels per spikelet of plant, relative*

r = .621 ± .024

For average yield of culm per plant and diameter of straw (Figs. 157 to 162), the correlation is highest in the medium condition of crowding, and lowest in the least condition of crowding, for both lines. In line 50 the highest and the lowest are .882 ± .009 and .765 ± .018,

Series 63

	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	Totals
1– 2	3	5	3		1	1						13
2– 3	2	2	2	1	2	1	1					11
3– 4			7	7	7	6	2					29
4– 5					5	9	9	1	2			26
5– 6					8	9	10	6	5	2		40
6– 7					3	7	19	7	6	2		44
7– 8						4	4	4	10	1	1	24
8– 9							5	6	6	1		18
9–10							2	6	2	7		17
10–11								2	2	2	3	9
11–12								2	2	1		5
12–13								1		1		2
Totals	5	7	12	8	26	37	52	35	35	17	4	238

FIG. 157.— *Correlation between average yield of culm per plant, in decigrams, subject; and diameter of straw, in decimillimeters, relative*

r = .765 ± .018

Series 100

	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	Totals
0–1	7	19	13	5	1	1							46
1–2		7	17	21	13	16	3						77
2–3				11	18	25	18	3	1				76
3–4					2	7	17	14	5				45
4–5							3	7	10	4			24
5–6							1		8	8	1		18
6–7									1	7	2	1	11
7–8											1		1
8–9											2		2
Totals	7	26	30	37	34	49	42	24	25	19	6	1	300

FIG. 158.— *Correlation between average yield of culm per plant, in decigrams, subject; and diameter of straw, in decimillimeters, relative*

r = .882 ± .009

Series 122

	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	Totals
0–1	1	3	16	34	18	5		1					78
1–2				5	16	47	18	8	2				96
2–3						2	23	31	15	1			72
3–4								8	18	7	3	1	37
4–5									4	5	2	1	12
5–6									1	3			4
6–7											1		1
Totals	1	3	16	39	34	54	41	48	40	16	6	2	300

FIG. 159.— *Correlation between average yield of culm per plant, in decigrams, subject; and diameter of straw, in decimillimeters, relative*
r = .866 ± .010

respectively. In line 62 the extremes are .878 ± .009 and .785 ± .015. The difference in each case, .117 ± .020 for line 50 and .093 ± .017 for line 62, is more than five times its probable error. This difference may

Series 68

	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	30–32	32–34	Totals
0– 1	3	1	1	2										7
1– 2		2	4	5	7	3								21
2– 3				1	5	14	4		1	1				26
3– 4					2	8	16	9	9	7	2			53
4– 5						2	7	18	15	11	2			55
5– 6							1	5	14	15	6	1		42
6– 7							1	5	7	12	8	7	1	41
7– 8							1	1	6	17	10	1	1	37
8– 9										4	5	3		12
9–10								1		2	2		1	6
Totals	3	3	5	8	14	27	30	39	52	69	35	12	3	300

FIG. 160.— *Correlation between average yield of culm per plant, in decigrams, subject; and diameter of straw, in decimillimeters, relative*
r = .785 ± .015

Series 102

	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	28–30	Totals
0–1	3	8	16	15	17	3	1							63
1–2				1	21	27	23	5	1					78
2–3						5	16	17	15	4	1	1		59
3–4								7	15	14	2	2		40
4–5								2	4	15	8	3		32
5–6										5	5	1	2	13
6–7										1	4	9		14
7–8														0
8–9												1		1
Totals	3	8	16	16	38	35	40	31	35	39	20	17	2	300

FIG. 161.— *Correlation between average yield of culm per plant, in decigrams, subject; and diameter of straw, in decimillimeters, relative*

r = .878 ± .009

be of some importance. It seems that, up to a certain point, as crowding increases the correlation between yield and diameter of straw increases, and that beyond this point there is a decrease in correlation.

Series 118

	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20	20–22	22–24	24–26	Totals
0–1	11	37	52	34	15	4						153
1–2				12	40	25	8			1		86
2–3						2	19	15	4			40
3–4								7	8			15
4–5									2	3		5
5–6											1	1
Totals	11	37	52	46	55	31	27	22	14	4	1	300

FIG. 162.— *Correlation between average yield of culm per plant, in decigrams, subject; and diameter of straw, in decimillimeters, relative*

r = .871 ± .009

Comparing average yield of culm per plant and breaking strength of straw (Figs. 163 to 168), it is seen that there is again a significant difference in correlation produced by crowding. In both lines the lowest

Series 63

	40– 60	60– 80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	220–240	240–260	260–280	280–300	300–320	320–340	340–360	360–380	380–400	400–420	420–440	440–460	520–540	Totals
1– 2	2	5	2	2		1	1																13
2– 3	1	2	1	1	2	2				2													11
3– 4		1	2	5	5	5	2	4	2	1			1		1								29
4– 5				2	3	1	1	3	3	3	4	4	1			1							26
5– 6					2	1	8	7	1	4	7	3	2		3	2							40
6– 7					1	1	4	10	6	4	7	1	2	3		2	1	1			1		44
7– 8							2	2	1	3	3	2	1	5	2	1	1		1				24
8– 9									3	2	7	1	1		3		1						18
9–10										1	5	3	2	1	1	2	1					1	17
10–11									1				2			1		2	1		2		9
11–12											1		3	1									5
12–13														1	1								2
Totals	3	8	5	10	13	11	18	26	17	20	34	14	15	11	11	9	4	3	2	0	3	1	238

FIG. 163.*— *Correlation between average yield of culm per plant, in decigrams, subject; and breaking strength of straw, in grams, relative*

r = .673 ± .024

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* See footnote to Fig. 73, page 93.

Series 100

	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	220–240	240–260	260–280	Totals
0–1	1	18	21	5	1										46
1–2			13	29	25	8	2								77
2–3				8	26	27	11	3	1						76
3–4					3	12	18	7	3	2					45
4–5							7	12	2	2	1				24
5–6							1	2	6	5	3		1		18
6–7									2	4	3	1	1		11
7–8									1						1
8–9												1		1	2
Totals	1	18	34	42	55	47	39	24	15	13	7	2	2	1	300

FIG. 164.— *Correlation between average yield of culm per plant, in decigrams, subject; and breaking strength of straw, in grams, relative*

r = .896 ± .008

correlation occurs when least crowding exists. In line 50, series 63, the coefficient is .673 ± .024, this being the lowest coefficient. The highest is .896 ± .008, for series 100, the difference being .223 ± .025, which is significant. In line 62 the extreme coefficients differ by .182 ± .022,

Series 122

	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	Totals
0–1	15	49	11	3								78
1–2		4	50	27	11	3	1					96
2–3				13	31	18	8	2				72
3–4					5	16	8	4	3		1	37
4–5						1	6	3		2		12
5–6							1	2		1		4
6–7								1				1
Totals	15	53	61	43	47	38	24	12	3	3	1	300

FIG. 165.— *Correlation between average yield of culm per plant, in decigrams, subject; and breaking strength of straw, in grams, relative*

r = .870 ± .009

Series 68

	0– 20	20– 40	40– 60	60– 80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	220–240	240–260	260–280	280–300	300–320	320–340	340–360	360–380	380–400	400–420	420–440	440–460	Totals
0– 1	1	1	3	1	1																			7
1– 2		1	1	5	7	3	3	1																21
2– 3			1	3	7	7	2	1	1		2		1		1									26
3– 4				1	2	3	11	7	8	4	3	7	2	2	3									53
4– 5					1		4	7	6	11	7	7	6	4	2									55
5– 6							2	1	5	3	4	12	5	4	1	2	1	1	1					42
6– 7								1	4		6	4	5	5	4	6	1	1	1	2			1	41
7– 8							2				4	8	7	4	7	3	1			1				37
8– 9											3	2		1			5	1						12
9–10									1			1	1					1	1		1			6
Totals	1	2	5	10	18	13	24	18	25	18	29	41	27	20	18	11	8	4	3	3	1	0	1	300

FIG. 166.—*Correlation between average yield of culm per plant, in decigrams, subject; and breaking strength of straw, in grams, relative*

$r = .701 \pm .020$

Series 102

	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	220–240	240–260	260–280	Totals
0–1	22	33	8												63
1–2		8	34	30	5		1								78
2–3			3	23	16	12	4	1							59
3–4				1	10	10	12	3	1	1	1	1			40
4–5					1	8	11	4	5	2	1				32
5–6				1		1	4	3		1		1	1	1	13
6–7							1	5	4	2	2				14
7–8															0
8–9									1						1
Totals	22	41	45	55	32	31	33	16	11	6	4	2	1	1	300

FIG. 167.— *Correlation between average yield of culm per plant, in decigrams, subject; and breaking strength of straw, in grams, relative*

r = .863 ± .010

which is also significant. In this line the greatest correlation is found with the greatest crowding. It appears, then, that there is an increase in correlation with increase in crowding, but that the limit is reached more quickly in line 50.

Series 118

	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180	Totals
0–1	39	78	30	6						153
1–2		4	24	37	19	1	1			86
2–3			1	6	9	16	8			40
3–4						3	5	7		15
4–5							1	2	2	5
5–6							1			1
Totals	39	82	55	49	28	20	16	9	2	300

FIG. 168.— *Correlation between average yield of culm per plant, in decigrams, subject; and breaking strength of straw, in grams, relative*

r = .883 ± .009

Average yield of culm per plant is correlated with average weight of straw per culm of plant (Figs. 169 to 174) in line 50 as follows: .901 ± .007 in series 122; .919 ± .007 in series 63; and .941 ± .004 in series 100. The difference of the extremes is here .040 ± .008, which is not highly

Series 63

	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	17–18	Totals
1– 2	2	11															13
2– 3		5	2	3		1											11
3– 4		2	14	8	5												29
4– 5				11	9	5	1										26
5– 6				4	14	12	10										40
6– 7					4	21	12	4	3								44
7– 8						2	5	12	5								24
8– 9							3	7	5	3							18
9–10								3	3	7	4						17
10–11									4	1	2	1	1				9
11–12										2	2				1		5
12–13										1						1	2
Totals	2	18	16	26	32	41	31	26	20	14	8	1	1	0	1	1	238

FIG. 169.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative*

r = .919 ± .007

Series 100

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	Totals
0–1	4	32	10										46
1–2		13	40	24									77
2–3			8	36	24	7	1						76
3–4				1	20	18	6						45
4–5						4	12	8					24
5–6							1	9	6	2			18
6–7								1	5	4		1	11
7–8										1			1
8–9										1		1	2
Totals	4	45	58	61	44	29	20	18	11	8	0	2	300

FIG. 170.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative*

r = .941 ± .004

Series 122

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	Totals
0–1	22	51	5							78
1–2		29	53	11	2		1			96
2–3			6	53	11	2				72
3–4				6	22	9				37
4–5					1	3	7	1		12
5–6						1	1	2		4
6–7									1	1
Totals	22	80	64	70	36	15	9	3	1	300

FIG. 171.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative*

r = .901 ± .007

significant. In line 62 the coefficients are, in order of value: .884 ± .008 in series 68; .888 ± .008 in series 118; and .944 ± .004 in series 102. The difference of the extremes is here .060 ± .009, which is probably of some significance. From these results it is evident that yield and weight of straw are highly correlated in the culms of oat plants. This correlation

Series 68

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	Totals
0– 1	1	3	3												7
1– 2		1	13	6	1										21
2– 3			5	12	8	1									26
3– 4				13	21	17	2								53
4– 5					14	22	17	2							55
5– 6						11	18	12	1						42
6– 7						3	13	9	11	4			1		41
7– 8							4	11	14	6	1			1	37
8– 9							2		4	4		1	1		12
9–10									1	3	1	1			6
Totals	1	4	21	31	44	54	56	34	31	17	2	2	2	1	300

FIG. 172.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative*

r = .884 ± .008

Series 102

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	Totals
0–1	19	39	5										63
1–2		15	51	11	1								78
2–3			4	37	17			1					59
3–4				1	19	14	6						40
4–5					2	11	13	5	1				32
5–6							6	4	1	1	1		13
6–7							1	2	7	4			14
7–8													0
8–9												1	1
Totals	19	54	60	49	39	25	26	12	9	5	1	1	300

FIG. 173.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative*

$r = .944 \pm .004$

is increased to a small extent when plants are grown in more crowded conditions up to a certain limit, beyond which the correlation is again reduced.

Series 118

	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	Totals
0–1	60	84	8	1					153
1–2		25	48	13					86
2–3			7	28	5				40
3–4					10	3	2		15
4–5						4	1		5
5–6								1	1
Totals	60	109	63	42	15	7	3	1	300

FIG. 174.— *Correlation between average yield of culm per plant, in decigrams, subject; and average weight of straw per culm of plant, in decigrams, relative*

$r = .888 \pm .008$

Interesting conditions are found when diameter of straw is correlated with breaking strength of straw (Figs. 175 to 180). The highest correlation in line 50 is in the most crowded condition, where the coefficient

Series 63

	40– 60	60– 80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	220–240	240–260	260–280	280–300	300–320	320–340	340–360	360–380	380–400	400–420	420–440	440–460	520–540	Totals
14–16	2	2	1																				5
16–18		4	1	2																			7
18–20		1	2	2	6	1																	12
20–22		1		3	1	2	1																8
22–24				2	3	6	3	10	1	1													26
24–26	1		1	1	1	2	10	7	6	3	2	2	1										37
26–28					2		3	6	6	13	10	5	2		3	2							52
28–30							1	2	3	1	12	2	4	5	2	1	1				1		35
30–32								1	1	2	9	4	5	3	3	2	2	2			1		35
32–34											1	1	2	3	3	3	1		1		1	1	17
34–36													1			1		1	1				4
Totals	3	8	5	10	13	11	18	26	17	20	34	14	15	11	11	9	4	3	2	0	3	1	238

FIG. 175.*— *Correlation between diameter of straw, in decimillimeters, subject; and breaking strength of straw, in grams, relative*

r = .820 ± .014

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* See footnote to Fig. 73, page 93.

Series 100

	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	220–240	240–260	260–280	Totals
6–8	1	4	2												7
8–10		8	8	8	2										26
10–12		5	11	6	6	2									30
12–14		1	10	11	8	5		2							37
14–16			2	7	12	10	3								34
16–18			1	9	14	12	12		1						49
18–20				1	11	14	11	4	1						42
20–22					2	4	7	9	1	1					24
22–24							6	8	7	2	2				25
24–26								1	3	7	5	1	2		19
26–28									2	2		1		1	6
28–30										1					1
Totals	1	18	34	42	55	47	39	24	15	13	7	2	2	1	300

FIG. 176.— *Correlation between diameter of straw, in decimillimeters, subject; and breaking strength of straw, in grams, relative*
r = .852 ± .011

is .913 ± .006. From this the decrease is graduated to the least crowded condition, where the coefficient is .820 ± .014. The difference is .093 ± .015, which is about six times its probable error. In line 62 the correlations are not graduated in the same order. The lowest again occurs in the

Series 122

	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	Totals
2–4	1											1
4–6	3											3
6–8	7	9										16
8–10	4	30	4	1								39
10–12		10	20	4								34
12–14		3	30	19	2							54
14–16			7	14	16	4						41
16–18		1		5	22	14	6					48
18–20					7	19	9	4	1			40
20–22						1	9	5		1		16
22–24								3	2	1		6
24–26										1	1	2
Totals	15	53	61	43	47	38	24	12	3	3	1	300

FIG. 177.— *Correlation between diameter of straw, in decimillimeters, subject; and breaking strength of straw, in grams, relative*
r = .913 ± .006

Series 68

	0– 20	20– 40	40– 60	60– 80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	220–240	240–260	260–280	280–300	300–320	320–340	340–360	360–380	380–400	400–420	420–440	440–460	Totals
8–10	1	1	1																					3
10–12		1	1	1																				3
12–14			1	3	1																			5
14–16			1		4	2	1																	8
16–18			1	3	6	1	2	1																14
18–20				3	4	6	8	5	1															27
20–22					1	4	6	5	7	2	4	1												30
22–24					2		3	5	12	8	4	3		1	1									39
24–26							2	1	4	5	10	14	9	3	3	1								52
26–28							2	1	1	3	6	17	12	10	8	6	1	2						69
28–30											5	6	6	5	5	1	3	1	2	1				35
30–32														1	1	3	4	1	1				1	12
32–34																				2	1			3
Totals	1	2	5	10	18	13	24	18	25	18	29	41	27	20	18	11	8	4	3	3	1	0	1	300

FIG. 178.— *Correlation between diameter of straw, in decimillimeters, subject; and breaking strength of straw, in grams, relative*

r = .871 ± .009

Series 102

	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180	180–200	200–220	220–240	240–260	260–280	Totals
4–6	3														3
6–8	7	1													8
8–10	10	6													16
10–12	1	14	1												16
12–14	1	14	20	3											38
14–16		6	15	14											35
16–18			7	23	8	2									40
18–20			1	11	11	4	4								31
20–22			1	3	9	13	7	2							35
22–24				1	3	11	16	5	2	1					39
24–26					1	1	4	7	4	2	1				20
26–28							2	2	5	3	3	1	1		17
28–30												1		1	2
Totals	22	41	45	55	32	31	33	16	11	6	4	2	1	1	300

FIG. 179.— *Correlation between diameter of straw, in decimillimeters, subject; and breaking strength of straw, in grams, relative*
r = .960 ± .003

least crowded condition, but the highest is in the medium condition of crowding. The difference is here .089 ± .009, which is great enough to be of significance. The diameter of straw is, then, highly correlated with breaking strength, and the lowest correlation occurs when crowding is least.

Series 118

	0–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180	Totals
4–6	11									11
6–8	22	15								37
8–10	6	40	6							52
10–12		22	21	2	1					46
12–14		5	21	24	5					55
14–16			6	13	10	1	1			31
16–18			1	7	8	7	4			27
18–20				2	3	8	6	3		22
20–22					1	4	3	4	2	14
22–24				1			1	2		4
24–26							1			1
Totals	39	82	55	49	28	20	16	9	2	300

FIG. 180.— *Correlation between diameter of straw, in decimillimeters, subject; and breaking strength of straw, in grams, relative*
r = .899 ± .007

When average weight of kernels is concerned in correlation, there is always the lowest correlation for the plants grown in the least crowded condition. There is usually, but not always, the greatest correlation in plants grown under the most crowded conditions. The differences, too, are usually of significant value. When average weight of kernels per plant is correlated with average number of kernels per culm of plant (Figs. 181 to 186), it is seen that in line 50 the extreme coefficients are .133 ± .043 for series 63, and .466 ± .030 for series 122 — a difference of .333 ± .052, which is more than six times its probable error. In line 62 the extreme coefficients are .288 ± .036 for series 68, and .487 ± .030 for series 118. The difference here is .199 ± .047, which is more than four times its probable error.

Series 63

	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	68–72	Totals
9–10			1		1												2
10–11																	0
11–12		2															2
12–13		1	1	1		1					1						5
13–14	1	1		2	1		2										7
14–15	2	2	1	1	7	4	4	3	1	1	1			1			28
15–16	2	1	1	5	1	3	5	1	4	2	1	2					28
16–17	2	1	1	4	2	2	5	6	5	2	1					1	32
17–18			2	2	1	6	3	5	4	1	3	1					28
18–19		2	1		2	2	7	1	3	2	3	1					24
19–20			2		8		2	2	2	3	2	2					23
20–21		2	1	1		3	1	1	3		3	2	1				18
21–22		1		1	1	4		2		4			1				14
22–23	1			2	2	1	1	1	2	1	1						12
23–24		1		1	2	2				1							7
24–25				1	1		2										4
25–26					1			1									2
26–27					2												2
Totals	8	14	11	21	32	28	32	23	24	17	16	8	2	1	0	1	238

FIG. 181.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative*

r = .133 ± .043

Series 100

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	Totals
4–5	1											1
5–6												0
6–7	1	1										2
7–8		1										1
8–9	1	1	1									3
9–10		7	3									10
10–11	1	9	2									12
11–12	1	10	3	5	1		2					22
12–13		8	5	4	2	4	1					24
13–14		5	10	6	4		1	2		1		29
14–15		5	10	9	8	8	4	4	2	2	2	54
15–16			6	11	7	5	7	4	2	3		45
16–17		1	6	4	5	2	1	3	1	1		24
17–18			2	8	3	3		3	2	1		22
18–19		3	5	5	3	2		2		1	1	22
19–20		3	2	5	2	2	1		2			17
20–21				2		1	2					5
21–22			2									2
22–23								1		1		2
23–24			1									1
24–25												0
25–26							1					1
26–27												0
27–28				1								1
Totals	5	54	58	60	35	27	20	19	9	10	3	300

FIG. 182.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative*

r = .363 ± .034

Series 122

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	Totals
6–7	1								1
7–8	5								5
8–9	4	4							8
9–10	6	3							9
10–11	5	6							11
11–12	5	12	3	1	1				22
12–13		7	6	4		3			20
13–14	3	7	6	4	1	1	1		23
14–15	3	7	4	3	3		2		22
15–16	1	7	8	7	6	1	1	1	32
16–17		9	6	7	3	6		1	32
17–18		6	13	9	3	3	3		37
18–19		9	10	6	7	1	2		35
19–20	1	1	2	10	3	1	2		20
20–21			4	1	1		3		9
21–22			1	6					7
22–23			1	1		1			3
23–24		1		1			1		3
30–31			1						1
Totals	34	79	65	60	28	17	15	2	300

FIG. 183.*—*Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative*

r = .466 ± .030

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* See footnote to Fig. 73, page 93.

Series 68

	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	60–64	64–68	Totals
8–9	2	1	2		1												6
9–10	1	1	2		2	1	1				1						9
10–11	1			3	3	1	4	4	3	1	3						23
11–12	1	1	6	2	2	4	4	1	6	3		4	1	1	2		38
12–13		1	2	1	3	6	9	8	6	4	5	3	4	2	3		57
13–14		2	1	2	2	4	10	3	8	10	12	5	4	2	1		66
14–15		2	1		1	7	1	7	5	7	2	4	7	2	4		50
15–16				3	2	2	1	3	2	5	7	2	4	1		1	33
16–17				2	2	2	2		1		2			1			12
17–18						1				2	2						5
18–19					1												1
Totals	5	8	14	13	19	28	32	26	31	32	34	18	20	9	10	1	300

FIG. 184.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative*

r = .288 ± .036

Series 102

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	40–44	44–48	48–52	52–56	56–60	Totals
3– 4	4	4														8
4– 5	3	3														6
5– 6	4	3	1													8
6– 7	2	2	1				1									6
7– 8	1	2	2	3												8
8– 9	1	2	6	2	1	2				1						15
9–10	1	3	7	5	9	6	2		1							34
10–11	1	8	4	6	3	7	4	7	5	4	2		1			52
11–12		1	8	10	6	7	4	6	3	4	4	2	1		1	57
12–13		3	5	6	6	5	8	1	1	2	1	4	1	3		46
13–14			8	4		2	3	3	6	2	3	1	1		1	34
14–15			2	1	4			3	1		3	1				15
15–16		1	1	1	1		2			2	1					9
16–17						1										1
17–18						1										1
Totals	17	32	45	38	30	31	24	20	17	15	14	8	4	3	2	300

FIG. 185.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative*

r = .471 ± .030

Series 118

	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	36–40	Totals
3– 4	1										1
4– 5	3										3
5– 6	6	2									8
6– 7	12	7									19
7– 8	9	13	3								25
8– 9	9	13	5	1		1		1			30
9–10	5	16	12	1	1						35
10–11	3	15	11	3	2	4	3	3			44
11–12	3	13	7	10	6	3	2	3	1		48
12–13	1	6	12	8	4	3	3	1	2		40
13–14		3	10	5	3	2		1		1	25
14–15		1	5	2	3	1		1			13
15–16			3	1				1			5
16–17			1		1	1					3
20–21		1									1
Totals	52	90	69	31	20	15	8	11	3	1	300

FIG. 186.*— *Correlation between average weight of kernels per plant, in milligrams, subject; and average number of kernels per culm of plant, relative*

r = .487 ± .030

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* See footnote to Fig. 73, page 93.

For average weight of kernels per plant correlated with average height of plant (Figs. 187 to 192), the extreme coefficients are, in line 50, .380 ± .037 for series 63, and .718 ± .019 for series 122 — a difference of .338 ± .042, which is about eight times its probable error. In line 62 the extreme coefficients are .450 ± .031 for series 68, and .733 ± .018 for series 102 — a difference of .283 ± .036, or about eight times its probable error.

Series 63

	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	Totals
9–10					1	1						2
10–11												0
11–12	1		1									2
12–13		1	1	1			2					5
13–14			1	2		2	1	1				7
14–15			2	1	4	13	4	2	2			28
15–16				4	4	6	6	4	4			28
16–17	1		1	2	4	5	7	8	2	2		32
17–18				3	4	6	6	5	2	1	1	28
18–19			2	3	1		8	3	6	1		24
19–20				1	2	5	4	4	2	4	1	23
20–21			2		1		3	4	3	4	1	18
21–22				1	1	2	1	4	1	3	1	14
22–23			1		1		3	2	3	2		12
23–24					1	1	1	2	1		1	7
24–25					1		1	1		1		4
25–26								1	1			2
26–27							1			1		2
Totals	2	1	11	18	25	41	48	41	27	19	5	238

FIG. 187.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative*

r = .380 ± .037

Series 100

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	Totals
4– 5		1											1
5– 6													0
6– 7		1		1									2
7– 8		1											1
8– 9		1	1	1									3
9–10		1	4	4	1								10
10–11		3	7	2									12
11–12	1	3	2	5	4	4	2	1					22
12–13			3	4	5	5	5	2					24
13–14		1	1	1	9	6	7	3	1				29
14–15			3	1	6	10	7	14	10	2	1		54
15–16				1	3	7	7	11	12	4			45
16–17			1		2	5	3	6	4	3			24
17–18					1	2	3	7	4	3	2		22
18–19					1	7	3	3	3	2	3		22
19–20				1	2	1	2	3	4	4			17
20–21								2		2	1		5
21–22						1		1					2
22–23												2	2
23–24								1					1
24–25													0
25–26										1			1
26–27													0
27–28										1			1
Totals	1	12	22	21	34	48	39	54	38	22	7	2	300

FIG. 188.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative*

r = .651 ± .022

Series 122

	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	Totals
6– 7		1										1
7– 8		3	1	1								5
8– 9		3	5									8
9–10			5	3	1							9
10–11		2	3	4	1	1						11
11–12		1	6	4	7	3		1				22
12–13			2	2	6	4	4	2				20
13–14			1	4	5	6	3	2	1	1		23
14–15			2	1	5	5	6	1	1	1		22
15–16	2				3	9	5	6	6		1	32
16–17					3	6	12	4	6	1		32
17–18					4	3	11	4	13	2		37
18–19					2	8	6	12	5	1	1	35
19–20					1	2	1	9	3	3	1	20
20–21							2	3	1	2	1	9
21–22								2	5			7
22–23									2	1		3
23–24					1				1		1	3
30–31										1		1
Totals	2	10	25	19	39	47	50	46	44	13	5	300

FIG. 189.*— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative*

r = .718 ± .019

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*See footnote to Fig. 73, page 93.

Series 68

	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	Totals
8– 9	1		2	1	1	1								6
9–10	1		2	1	1	4								9
10–11		1			3	10	8		1					23
11–12		2	1	2	5	7	5	10	2	3		1		38
12–13		1		3	4	13	9	15	6	5	1			57
13–14			1	2	4	9	14	14	11	9	2			66
14–15			1	2	1	4	9	13	7	8	1	3	1	50
15–16			1	1	1	2	2	6	7	6	6	1		33
16–17				1	1		4	1	2		3			12
17–18								2			3			5
18–19						1								1
Totals	2	4	8	13	21	51	51	61	36	31	16	5	1	300

FIG. 190.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative*

r = .450 ± .031

Series 102

	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	Totals
3– 4		1	3	1	2	1									8
4– 5	1	1	1	3											6
5– 6				6	1	1									8
6– 7					3	2			1						6
7– 8				3		2	3								8
8– 9			1		3	3	5	2			1				15
9–10			1		1	7	4	8	10	3					34
10–11					2	3	8	8	9	9	9	2	1	1	52
11–12						2	5	5	14	13	7	10	1		57
12–13						1	3	5	10	10	8	5	3	1	46
13–14							1	4	9		6	8	5	1	34
14–15								1	2	3	1	3	4	1	15
15–16									3		4	1	1		9
16–17												1			1
17–18												1			1
Totals	1	2	6	13	12	22	29	33	58	38	36	31	15	4	300

FIG. 191.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative*

r = .733 ± .018

Series 118

	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	Totals
3– 4	1												1
4– 5	1	1	1										3
5– 6		1	6	1									8
6– 7	2	5	5	4	3								19
7– 8		1	8	8	8								25
8– 9		2	2	9	9	3	4		1				30
9–10			1	7	7	15	5						35
10–11				3	11	10	8	6	3	3			44
11–12		1		3	7	5	8	14	6	2	2		48
12–13					5	9	6	6	11	3			40
13–14						5	5	9	2	3	1		25
14–15							3	4	3	2		1	13
15–16						1	3				1		5
16–17							1	1	1				3
20–21							1						1
Totals	4	11	23	35	50	48	44	40	27	13	4	1	300

FIG. 192.*— *Correlation between average weight of kernels per plant, in milligrams, subject; and average height of plant, in centimeters, relative*

r = .714 ± .019

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* See footnote to Fig. 73, page 93.

For average weight of kernels per plant correlated with average length of head per plant (Figs. 193 to 198), in line 50, for series 63 the coefficient is .081 ± .043 and for series 122 it is .535 ± .028. The difference here is .454 ± .051, which is about nine times its probable error. In line 62 the extreme difference is .341 ± .044, which is nearly eight times its probable error.

Series 63

	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	16–17	Totals
9–10				1			1					2
10–11												0
11–12		2										2
12–13				1	1	2				1		5
13–14			1	2		3	1					7
14–15	2		3		3	7	5	2	4	1	1	28
15–16		2	1	3		7	4	7	2	2		28
16–17	1	1	2	4	2	1	5	9	6	1		32
17–18				2	1	8	5	7	3	2		28
18–19			2	2	2	1	6	5	3	3		24
19–20	1			1	3	5	3	7	1	2		23
20–21			2		2	2	3	1	3	4	1	18
21–22		1		1	2		4	3	1	1	1	14
22–23	1		1	1	2		2	3	1	1		12
23–24			1	1	2	1	1			1		7
24–25			1	1			2					4
25–26					1			1				2
26–27						2						2
Totals	5	6	14	20	21	39	42	45	24	19	3	238

FIG. 193.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average length of head per plant, in centimeters, relative*

$r = .081 \pm .043$

Series 100

	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	Totals
4–5	1												1
5–6													0
6–7	1		1										2
7–8		1											1
8–9		1		1	1								3
9–10		2	3	2	3								10
10–11		3	4	3	1	1							12
11–12		4	2	6	2	3	3	2					22
12–13			4	3	3	6	4	3	1				24
13–14			1	5	8	3	8		2	2			29
14–15			2	4	6	15	8	6	6	3	4		54
15–16				2	3	7	14	8	8	2		1	45
16–17				2	4	9	3		2	2		2	24
17–18					3	6	5	3	3	2			22
18–19			1	3	3	6	3	3		2	1		22
19–20				2	3	6	3		2	1			17
20–21						1	3	1					5
21–22				1		1							2
22–23									2				2
23–24						1							1
24–25													0
25–26								1					1
26–27													0
27–28						1							1
Totals	2	11	18	34	40	66	54	27	26	14	5	3	300

FIG. 194.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average length of head per plant, in centimeters, relative*

$r = .408 \pm .032$

Series 122

	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	Totals
6– 7	1											1
7– 8		4	1									5
8– 9		4	3	1								8
9–10		2	4	2	1							9
10–11		2	5	4								11
11–12		2	6	6	6	1	1					22
12–13			1	5	1	7	3	1	2			20
13–14			5		7	5	2	2	1	1		23
14–15		1	2	4	4	3	3	3		2		22
15–16	1			4	4	8	8	2	2	3		32
16–17				5	6	7	4	6	2	2		32
17–18				4	4	10	8	8	2	1		37
18–19				4	6	7	10	4	3	1		35
19–20				2		6	6	2	4			20
20–21						3	2	1	2	1		9
21–22					1	3	3					7
22–23							2		1			3
23–24				1		1		1				3
30–31					1							1
Totals	2	15	27	42	41	61	52	30	19	11	0	300

FIG. 195.*— *Correlation between average weight of kernels per plant, in milligrams, subject; and average length of head per plant, in centimeters, relative*

r = .535 ± .028

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*See footnote to Fig. 73, page 93.

Series 68

	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	15–16	Totals
8– 9	2	1		1	2							6
9–10	1		2	2	1	2	1					9
10–11		1	1	2	3	5	6	2	3			23
11–12	1	1	3	2	5	10	8	4	3		1	38
12–13		1	1		12	11	10	14	5	3		57
13–14		1	2	1	9	4	22	19	6	1	1	66
14–15			3	3	3	8	12	8	7	5	1	50
15–16			1	1	5	3	7	9	7			33
16–17				1	4	2	2		2	1		12
17–18				1			3		1			5
18–19				1								1
Totals	4	5	13	15	44	45	71	56	34	10	3	300

FIG. 196.— *Correlation between average weight of kernels per plant, in milligrams, subject; and average length of head per plant, in centimeters, relative*

r = .265 ± .036

Series 102

	2– 3	3– 4	4– 5	5– 6	6– 7	7– 8	8– 9	9–10	10–11	11–12	12–13	13–14	Totals
3– 4	3	1	2	2									8
4– 5	1	1	4										6
5– 6		1	6		1								8
6– 7		1	1	2		1	1						6
7– 8		1	1	1	2	1	2						8
8– 9	1		2	1	4	3	1	1	1			1	15
9–10		1	1	1	7	7	7	9	1				34
10–11			2	7	4	6	4	11	12	5	1		52
11–12			1	1	9	8	6	12	10	8	2		57
12–13			1	2	4	3	11	9	9	5	2		46
13–14				2	5	3	7	6	3	7	1		34
14–15					2	2	2	3	4	2			15
15–16				1	1		2	3		1	1		9
16–17							1						1
17–18							1						1
Totals	5	6	21	20	39	34	45	54	40	28	7	1	300

FIG. 197.—*Correlation between average weight of kernels per plant, in milligrams, subject; and average length of head per plant, in centimeters, relative*

r = .535 ± .028

Series 118

	1– 2	2– 3	3– 4	4– 5	5– 6	6– 7	7– 8	8– 9	9–10	10–11	11–12	Totals
3– 4		1										1
4– 5		2	1									3
5– 6		2	4	2								8
6– 7	1	2	8	6	1	1						19
7– 8		3	5	6	9		2					25
8– 9	1	2	5	4	11	4	1		1	1		30
9–10		1	3	4	10	11	6					35
10–11			2	3	12	11	4	5	4	3		44
11–12		1	3		8	8	12	8	8			48
12–13				1	7	8	12	8	3	1		40
13–14				1	1	8	8	4	2		1	25
14–15						2	7	3	1			13
15–16						2	2		1			5
16–17						1	1			1		3
20–21					1							1
Totals	2	14	31	27	60	56	55	28	20	6	1	300

FIG. 198.*—*Correlation between average weight of kernels per plant, in milligrams, subject; and average length of head per plant, in centimeters, relative*

r = .606 ± .025

---

*See footnote to Fig. 73, page 93.

There is a decided increase, then, in the correlation of average weight of kernels per plant with height of plant, length of head, and average number of kernels per culm, as plants are crowded together more closely in the row. The same condition exists also when average weight of kernels is correlated with average yield of culm per plant, as noted above. In line 62, however, in two cases (with yield and with height of plant) the limit is reached at the medium condition of crowding, beyond which there is a decrease in correlation.

From the foregoing it is seen that different degrees of crowding in the row may produce striking differences in the correlation between certain characters of plants in the same pure line of oats.

The differences between the correlation coefficients when the same characters are correlated in line 50 range from a minimum of .024 ± .009 to a maximum of .514 ± .053. In line 62 the range of differences is from .021 ± .010 to .561 ± .046. The smallest range was obtained when average yield of culm per plant was correlated with average number of kernels per culm of plant in one case and with average number of spikelets per culm of plant in the other case, and the greatest range in both cases was obtained when the former character was correlated with average number of kernels per spikelet of plant.

Whenever the average weight of kernels per plant is correlated with another character there is always a considerable difference produced in correlation by crowding. In every case when this factor has been used, the lowest correlation has been found in conditions of least crowding. The greatest correlation has usually been found in the condition of greatest crowding. In two cases, however, both in line 62, the greatest correlation is found in the intermediate condition, these cases being, respectively, with yield and with height of plant. It will be remembered that the crowding in series 118 is the greatest that is dealt with, being considerably more than that in series 122 (Table 11, page 154). There appears, then, to be a point beyond which certain correlations decrease.

The correlation between yield and other characters is changed by more than .050 in six cases out of nine in both lines dealt with: in line 50 the six relative characters are height, length of head, weight of kernels, diameter of straw, number of kernels per spikelet, and breaking strength of straw; in line 62 weight of straw is substituted for length of head, the other characters being the same.

Yield increases slightly in its correlation with height as crowding increases up to a certain point, beyond which there is a gradual decrease in correlation. When yield is correlated with length of head, the differences are so small that definite conclusions cannot be drawn. Degrees of crowding have little effect on the correlation between yield and number of kernels. The same is true for yield and number of spikelets. There is a slight tendency shown for the correlation between yield and average weight of kernels to increase as crowding increases. There is a decided and significant increase in correlation between yield and number of kernels per spikelet as crowding increases. It seems that, up to a certain point, as crowding increases the correlation between yield and diameter of straw increases, and that beyond this point there is a decrease. There is an increase in correlation between yield and breaking strength of straw with increase in crowding, but a limit is reached, beyond which there is a decrease. Yield and weight of straw are highly correlated in the culms of oat plants, and the correlation is increased to a slight extent by crowding up to a certain point, beyond which there is a decrease. The diameter of straw is highly correlated with the breaking strength, but the least correlation exists when the crowding is least.

In every case when significant differences exist in the correlations between characters in plants grown under different conditions of crowding, there is an increase in correlation produced by more crowded conditions. In no case is the coefficient of correlation for the least crowded condition the largest, although the differences are often very small. There is, however, sometimes a decrease in correlation beyond a certain degree of crowding, in which case the coefficient for the intermediate degree of crowding is the highest.

It has been shown by this study that environmental conditions may influence the degree of correlation of certain characters to a marked extent. Such conditions may make of no significance the so-called varietal and other differences obtained by several investigators.

GENERAL DISCUSSION AND CONCLUSION

This study of oats has been taken up under four heads, as was outlined at the beginning of the paper. Various data have been presented and discussed under each of these heads, and summaries and conclusions have been made at the end of each division. The study has been pursued with several purposes in view. One purpose has been to determine the averages, the amounts of variation, and the correlations that exist in various characters of oats. Another has been to determine the effects on these characters of various environmental influences. Under the latter head data on the question of methods to be pursued in biometrical work with cereals have been adduced.

Correlation has been carefully considered. This was deemed advisable because of the increasing use of, and attention given to, such studies. The correlations taken up have been principally of yield and average weight of kernels with various other characters. Webber (1906) has classified correlation into four groups — *environmental*, *morphological*, *physiological*, and *coheritai*. It is very difficult to place the correlations here considered in this classification. It is certain that no cases of coherital correlations have been shown. Some of the correlations would probably be classed as environmental. But it has been shown that change in environment often produces radical changes in the correlation of certain parts. Environmental correlation, then, is not so simple a thing as might be supposed. Between morphological and physiological correlation there is no clear distinction, and it is probable that all the correlations here dealt with could be classified under either head.

The classification given by East (1908) is simpler in its main divisions these being *somatic* and *gametic*, but the subdivisions are not yet distinctly defined. The correlations treated here are somatic, but a further classification according to the subdivisions given would be difficult and perhaps unprofitable.

Correlations have been classified by Love and Leighty (1914) as *fluctuating* and *stable*, these divisions being based on the behavior of the relationship of the characters concerned when variation occurs in environmental conditions, such as exist in different years or in different locations. As the names indicate, the correlations of the first class may be made to vary considerably by changes in conditions, while those of the second class remain of about the same value or are stable in character. Both these classes of correlations are found among those reported in this paper.

The first division of this study is a comparison of biometrical constants determined for oat plants and for the culms of the same plants. From the results obtained it may be concluded that, for statistical work with oats, practically the same means and correlation coefficients will be obtained whether plants are used as units or the culms of the same plants are used as units, but that these constants will be slightly greater for the latter method. The standard deviations and coefficients of variability will also be greater when culms are the units. The biometrical results obtained by the several investigators, whether they worked with culms or with plants, are, however, comparable, with slight reservation, as far as this factor affects the results.

The second division of this study is a biometrical comparison of varieties of oats. In this study, considerable difference is shown in average yield of culm per plant. This is due to the larger kernels produced by certain varieties, since the numbers of kernels and of spikelets are about the same for the different varieties. There are varietal differences in the height of culm. The average number of kernels per spikelet is greatest in the Sixty Day and smallest in the Early Champion variety. The proportion of straw to grain differs in the different varieties. Considerable difference is found in the amount of variability of different characters of the varieties. The greatest variability, in all characters but one, is found in the Welcome variety, while each of the others is least variable in one or more characters. The coefficients of correlation are usually fairly close together for the different varieties, but some differences occur that may be due to varietal causes.

The third division of this study is a comparison of biometrical constants determined for oat plants grown in hills and in drills. Regarding all characters here studied, the means are greater for plants grown in hills than for plants grown in drills. The least difference in the means occurs in the cases of average height of plant and average weight of kernels per plant. There is greater variability in average yield of culm per plant and average weight of straw in plants grown in hills, but much less variability for plants so grown in average height and average weight of kernels. The variability in number of kernels and number of spikelets is slightly greater for the plants grown in hills. Rather large differences occur in the same variety between the coefficients of correlation determined for the plants grown in the two ways. Whenever large differences in the coefficients of correlation occur, those for the plants grown in hills are always the smaller in amount. The differences due only to the growing condition may amount to more than any varietal differences observed in this work. The constants, then, obtained by different investigators are comparable only in so far as the conditions of growth are comparable.

The fourth division of this study deals with the effect of different degrees of crowding on biometrical constants of oats. Oat plants grown in very crowded conditions produce but one culm to a plant, but, as more room is given, more than one culm are produced by many plants. The development of plants in most characters is greater in less crowded than in more crowded conditions. Variability decreases with increase in crowding for yield, number of kernels, number of spikelets, and breaking strength of straw; but for height the least variability occurs when crowding is least. In every case when significant differences exist in the correlations between characters in plants grown under different conditions of crowding, there is an increase in correlations produced by more crowded conditions, but there is sometimes a decrease beyond a certain degree of crowding. It has been shown that environmental conditions may influence the degree of correlation of certain characters to a marked extent. Such conditions of environment may make of no significance the so-called varietal and other differences obtained by several investigators.

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CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

PHYSIOLOGICAL STUDIES OF BACILLUS RADICICOLA OF CANADA FIELD PEA

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PHYSIOLOGICAL STUDIES OF BACILLUS RADICICOLA OF CANADA FIELD PEA

PHYSIOLOGICAL STUDIES OF BACILLUS RADICICOLA OF CANADA FIELD PEA[1]

MARTIN J. PRUCHA

INTRODUCTION

Ever since the discovery that the formation of nodules on the roots of legumes is associated with a definite microorganism, there have appeared on the market pure cultures of this organism for the artificial inoculation of leguminous plants. Nobbe and Hiltner (1897)[2] first caused to be placed on the market a pure culture called nitragin,[3] in which the medium employed was gelatin. The efficiency of the culture was low, and Nobbe and Hiltner explained this by ascribing to the culture a loss of infecting power due to the medium used. Moore (1905) stated, as a result of his work, that the virulence of the organism was impaired when it was propagated on nitrogenous media, and consequently he used a nitrogen-free medium.

The same opinion has been held by various other investigators, and is observed by all firms and institutions that distribute pure cultures of the legume organism. Despite the fact that media low in nitrogen have been employed, the results with pure cultures have not always been satisfactory, and Kellerman (1912) has stated that the pure cultures have not been as efficient as field soil. It would seem, then, that the conditions of isolation and cultivation of the legume organism must influence the organism, or that possibly the soil conditions would exert some influence on the efficiency of the organism to effect inoculation or would have an influence on the plant to resist infection.

SCOPE OF THE INVESTIGATION

With these ideas in mind, the investigation herewith reported was undertaken. It is concerned with the following: (1) Isolation and identification of the organism causing nodule development on the roots of Canada field pea; (2) A study of the influence of various factors on nodule development in Canada field pea when the plant is grown in water or soil cultures; (3) A study of the influence of various environmental conditions on the infecting power of the organism.

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[1] Laboratory of Plant Physiology, Contribution No. 15.

[2] Dates in parenthesis refer to bibliography, page 79.

[3] This is not the nitragin now on the market.

METHOD OF INVESTIGATION

Organism

Bacillus radicicola from Canada field pea was used for all the experiments except number 11, for which the organism from alfalfa was used. The organism was isolated as described on page 14. The stock culture was kept in the laboratory on agar slopes of medium 335, and was exposed to diffused light during the entire investigation. It was transferred at irregular intervals of time varying from one to three months.

Media

The various kinds of media used are referred to in the text by their laboratory numbers. In table 1 are given the laboratory numbers and the composition of the principal media employed. In addition to these a number of other media were used, and these are described subsequently. All the media were prepared according to the recommendations on the Descriptive Chart of the Society of American Bacteriologists.

Sterilization of utensils

Strict precautions were observed in sterilizing all the utensils. Glassware was sterilized for two hours at from 150° to 170° C. Scalpels and similar instruments, which resist high temperature, were heated in the flame. For work requiring air free from dust, a room was used into which a stream of steam was allowed to flow until the air was saturated; after condensation of the steam, the air in the room was conspicuously free from any dust.

Sterilization of seed

The experiments in Part III required seed free from *B. radicicola.* It was necessary, therefore, to sterilize the seed. In experiments 10 and 11, 95-per-cent alcohol was used, the seed being treated for thirty seconds. In experiments 12 and 13 a solution of bleaching powder was used. The author is indebted for this method to Professor J. K. Wilson, of Cornell University. The solution is prepared by mixing 8 grams of bleaching

TABLE 1. LABORATORY NUMBERS, COMPOSITION, AND REACTION OF MEDIA

Laboratory number	Composition	Reaction
Medium 101	0.2 gram $MgSO_4$ 1 gram KH_2PO_4 0.3 gram $Ca(NO_3)_2$ 0.01 gram $FeCl_3$ 1000 cc. distilled water	Not changed
Medium 300	15 grams agar 3 grams Liebig's beef extract 10 grams Witte's peptone 1000 cc. distilled water	+1, Fuller's scale
Medium 310	Same as 300, plus 2 per cent dextrose	Neutral
Medium 331	15 grams agar 10 grams cane sugar 1 gram KH_2PO_4 0.2 gram $MgSO_4$	Not changed
Medium 334	15 grams agar 0.2 gram K_2HPO_4 0.2 gram $MgSO_4$ 0.2 gram $NaCl$ 0.2 gram $CaSO_4$ 0.2 gram $CaCO_3$ 1000 cc. tap water	Not changed
Medium 335	Same as 334, plus 20 grams cane sugar	Not changed
Medium 337	Same as 335, plus 10 grams Witte's peptone	Not changed
Medium 400	15 grams agar 3 grams Liebig's beef extract 20 grams Witte's peptone 1000 cc. distilled water	+1, Fuller's scale
Crone's solution	1 gram KNO_3 0.5 gram $Fe_3(PO_4)_2$ 0.25 gram $CaSO_4$ (gypsum) 2000 cc. distilled water	Not changed
Pfeffer's solution	4 grams $Ca(NO_3)_2$ 1 gram KNO_3 1 gram $MgSO_4$ 0.5 gram KCl 0.06 gram $FeCl_3$ 6000 cc. distilled water	Not changed

powder with 140 cubic centimeters of water; after standing for a few hours, the clear liquid is decanted and is then ready for use. Seed was kept for three hours in this solution. According to Professor Wilson's results, seed treated in this way is completely sterilized. This method of seed sterilization will later be published in detail.

That the methods of seed sterilization employed were effective was proved by a large number of experiments, and particularly by experiments with soy beans. The organism producing nodules in soy bean is not present in the soil of this region, and in all the experiments made not a single nodule developed in the cultures in which sterilized seed was used unless the plants were subsequently inoculated. Unsterilized seed occasionally produced plants with nodules.

Sterilization of media and soil

All the media used for pure cultures, if in test tubes of small volume, were sterilized in an autoclave for fifteen minutes at 120° C.

In experiments 10, 11, 12, and 13 it was necessary to sterilize the soil in which the plants were grown. Three-inch flowerpots and glass tumblers, each containing about 300 grams of soil, were used for this purpose. These were sterilized for three hours at 120° C., in a large canner's retort. This retort was found very useful, since several hundred of the flowerpots could be sterilized at one time.

Method of growing the plants

In experiments 1, 2, 3, 4, 5, and 6, the plants were grown in water cultures; in experiments 7, 8, 9, 10, 11, 12, and 13 they were grown in soil.

For the water cultures glass vessels were employed. The vessels were filled with the nutrient solution and the opening was covered with paraffin paper. The seed was then germinated in a moist chamber, and when the radicle was about three centimeters long it was inserted into the solution through a small hole in the paraffin paper, allowing the cotyledons to rest on top of the paper.

For the soil cultures flowerpots and glass tumblers were used. These were filled with sandy soil, were covered with paper, and, for experiments 10, 11, 12, and 13, were sterilized. The seed was planted directly in the soil, the paper covers being kept on until the seedlings began to push them off.

Distilled water was used for watering the plants in experiments 1, 2, 3, 4, 5, 6, 7, 8, and 9. In experiments 10, 11, 12, and 13, boiled tap water was used.

The plants in all the experiments were grown in the greenhouse. When special precautions were necessary to guard against contamination during the growing period, the plants were kept in an especially constructed culture room. For this purpose a part of the greenhouse was set off by a partition. Cracks in the walls and around the panes of glass were filled with plaster of paris. For ventilation, two panes of glass were replaced by a special frame fitted with a layer of cotton held between two pieces of cheesecloth.

Inoculations

Inoculations were made at the time of planting the seeds or within a day or two following. The culture of the organism to be used for inoculation was introduced into sterile water, and the infusion was then added to the soil or the water culture in which the plants were grown. In some experiments quantitative inoculation was made, in which a specific amount of the infusion was added.

Examination of plants for nodules

Nodules usually appeared in about two weeks. The plants were examined at the end of three weeks. The roots were washed and the nodules were counted, and a note was made of the size and the place of attachment of the nodules. Since the soil in the vicinity of Ithaca is well inoculated with the Canada field pea organism, plants kept longer than three weeks were subject to some contamination.

Special method for growing plants under sterile conditions

For certain experiments it was necessary to maintain absolutely sterile conditions throughout the period of experiment. The method employed was as follows: A large glass cylinder, 65 centimeters high and 10 centimeters in diameter, was used as a growth chamber. In the bottom of the cylinder a few pieces of broken flowerpot were placed and were just covered with water, and on the top of these was set a four-inch pot, filled with a sandy soil. The cylinder was plugged with cotton, through which was passed a glass tube 7 millimeters in diameter and 65 centimeters long, the lower end resting on the surface of the soil in the pot and the upper end protruding above the cotton plug. The tube was plugged at its upper end with cotton. The whole was sterilized in the autoclave for five hours at 15 pounds pressure.

The seeds were sterilized by the bleaching-powder method. The sterilized seed were dropped into the pots through the glass tube, and by manipulation of the tube they were buried in the soil. The soil of the pots was inoculated by introducing through the tube a few cubic centimeters of water containing the nodule-forming organisms.

PART I. ISOLATION AND IDENTIFICATION OF THE ORGANISM

ISOLATION OF BACILLUS RADICICOLA

On October 10, 1910, a field pea plant 30 centimeters high, with a great abundance of nodules, was procured. The whole plant was washed thoroughly in running water. One of the nodules, of firm consistency, was selected and cut off in such a way as to leave about 3 centimeters of the root on each side of the nodule; cut off in this way the nodule is more easily manipulated. The nodule was then disinfected in a solution of one part of formalin to forty parts of water, for five minutes. Four petri dishes were prepared, each containing a few drops of sterile water. The nodule, after being disinfected, was washed in sterile water, placed on a filter paper, and cut open, and with a pointed scalpel a part of the central tissue was removed and placed in petri dish 1. This nodule tissue was crushed and mixed with the water. Three loopfuls of this infusion was transferred from plate 1 to plate 2, three loopfuls from plate 2 to plate 3, and three loopfuls from plate 3 to plate 4. Ten cubic centimeters of medium 331 were then poured into each petri dish, and after sufficient agitation to effect equal distribution of the organisms the plates were allowed to incubate at 20° C. In three days a few colonies characteristic of the nodule organism became visible on plate 1; on the other plates plenty of colonies were present, but were visible only by the microscope. In ten days the small colonies became large enough to be conveniently transferred.

As far as could be ascertained from the general appearance of the colonies, all the plates contained only one organism. The large colonies that developed on plate 1 appeared to be giant colonies, having started from small pieces of the nodule tissue in which a large number of the organisms were held. In order to obtain a pure culture of this organism, one of the small colonies was introduced into 5 cubic centimeters of sterile water in a test tube, and from this infusion a number of petri dishes of different dilutions were again prepared. From one of the colonies that developed on these petri dishes a transfer was made on agar slope with medium 335. This culture was used in all the experiments except experiment 11. The stock culture was kept on a shelf in the laboratory, and consequently was exposed for nearly three years to diffused light and to the ordinary variations of temperature and other atmospheric changes. The transferring of the stock culture was made at irregular intervals of time varying from one to three months, and the cultures were kept in test tubes on agar slopes of medium 335.

IDENTIFICATION OF THE ORGANISM

In order to be certain that the organism isolated was the causal organism of the nodules on Canada field peas, the following procedure was followed: (1) Canada field peas were grown under sterile conditions and were inoculated with this organism; (2) from one of the nodules that developed under sterile conditions a culture, No. 2, was isolated by the same method as described above; (3) the original culture and the culture No. 2 were again tested as to their ability to cause the development of nodules on Canada field peas under sterile conditions; (4) the two cultures were compared in the laboratory with respect to their morphological characters and physiological activities.

Ten Canada field peas were grown under sterile conditions according to the method described on page 13, one plant in each of ten glass cylinders. Five of the plants were inoculated with the above organism, and five were left without inoculation, as controls. At the end of six weeks the plants were examined. They had made a fair growth, having reached a height of 60 centimeters. They were spindling, however, the leaves were small, and the root system was very poorly developed. All the inoculated plants had nodules on their roots, while the controls were free from any nodules. Each plant and the soil in which it was grown were examined for contamination. One of the controls was found to be contaminated with a mold, and one of the inoculated plants was contaminated with a yellow organism. Four of the controls were sterile, and four of the inoculated plants were found to be sterile with respect to organisms other than *B. radicicola.*

A pure culture of the organism was isolated from a nodule found on one of the plants grown under sterile conditions. This organism was again tested as to its ability to produce nodules on Canada field peas. Fifteen Canada field pea plants were grown under sterile conditions again, in a similar manner to that described above. Five were inoculated with this organism, five were inoculated with the original organism, and five were left as controls. Again all the plants inoculated with both the cultures developed nodules on their roots, while the controls had none. The organism that was isolated on October 10, 1910, therefore, was the causal organism of nodules on Canada field pea plants, and as far as could be determined by laboratory methods it was also a pure culture.

The two cultures of the organism — the one isolated originally, and the other isolated from a nodule of a plant grown under sterile conditions — were studied in the laboratory with respect to their morphology and their physiological activities. An exhaustive study of this phase was not undertaken, the study being carried only far enough to show whether the two cultures were the same organism. The study consisted in propagating the two cultures on various media and in comparing and describing their cultural and biochemical features. The descriptions were recorded on the Descriptive Chart adopted for such use by the Society of American Bacteriologists (1907).

Morphology of the organism

Bacillus radicicola of Canada field pea produces no spores when propagated on the artificial media in the laboratory. In a young culture on artificial media, the organism is in the form of small rods about one micron long. In this form it is able to multiply by fission, like other bacteria. Under certain conditions — for example, with the addition of certain nutrients, such as sugar, to the media — some of these small rods develop into large cells, which are generally called bacteroids. Some of the bacteroids assume the characteristic X and Y forms, the same as are found in the nodules. The development of the bacteroids seems to be largely a matter of nutrition, and the bacteroids are not a degenerate form, but a normal form, of the organism.

In a culture twenty-four hours old on an agar slope the organism is very motile. As the agar-slope culture gets older, fewer and fewer of the organisms show motility, until in a culture about two weeks old no motility is detected. Motility seems to be influenced by environmental conditions. This point has not been studied extensively, beyond the observation that on nitrogenous media the motility is lost sooner than on nitrogen-free media.

Since the generic name of the organism depends on the presence and the place of attachment of flagella, and since there has been so much uncertainty on this point, an effort was made to demonstrate the number and the arrangement of the flagella. Beyerinck (1890) was the first who claimed to have isolated the organism in a pure culture. He described it as having one polar flagellum, and named it *Bacillus radicicola.* In 1905, Moore, agreeing with Beyerinck as to the number of flagella and wishing to conform to Migula's classification, changed the generic name from *Bacillus* to *Pseudomonas.* Edwards and Barlow (1909) found only one long, whiplike flagellum, thus agreeing with Beyerinck and Moore. De' Rossi (1907) was the first investigator who found the organism of *Vicia faba* to have about eight flagella with a peritrichic arrangement. Zipfel (1912) agreed with de' Rossi, stating that the organism has numerous flagella. In 1912 Kellerman, using a special method, also succeeded in staining the organism of several leguminous plants. He likewise found the organism to have several flagella peritrichically arranged.

In this investigation the following method was used for staining the organism of Canada field pea for flagella:

An agar slope culture twenty hours old, on medium 335 at 24° C., was very carefully transferred into 3 cubic centimeters of sterile distilled water in a test tube. This was allowed to stand for about four hours at a constant temperature, without being shaken or disturbed in any way. A drop of this infusion was placed on a cover glass and allowed to dry at room temperature, and when dry it was stained.

It was essential that the cover glasses should be clean. To this end they were treated with a cleaning solution of potassium bichromate and sulfuric acid, washed in water, placed in alcohol, and finally dried with a piece of cheesecloth which had been treated with ether in order to get rid of any fats. The cover glasses were then placed in a petri dish and baked in the oven for three hours at 200° C. If cover glasses are treated in this way a large amount of difficulty in the staining of flagella is avoided.

Pitfield's mordant as modified by Muir, and carbol fuchsin, were used for staining. The mordant has the following composition:

	Cubic centimeters
Tannic acid, 10 per cent aqueous solution	10
Corrosive sublimate, saturated aqueous solution	5
Alum, saturated aqueous solution	5
Carbol fuchsin	5

The mordant was applied for six minutes and the preparation was then very thoroughly washed with water. Carbol fuchsin was then applied for nine minutes.

It was found that the organism from Canada field pea has peritrichic arrangement of flagella. The largest number of flagella observed was six, arising from any part of the organism, and the indications were that the organism may have an even larger number. According to Migula's classification the organism is *Bacillus*.

Cultural and biochemical features

The surface colonies in a petri dish on agar medium 335 are colorless, watery, and very viscous. When ten days old, at 20° C., they are about 3 millimeters in diameter, although occasionally colonies 10 millimeters in diameter may develop. The colonies under the surface are invariably of spindle shape. Under the 16-millimeter objective the microscopic structure appears granular.

On agar slope with peptone and beef extract, the growth is watery, scanty, and colorless at first; after long standing it becomes brownish. In standard gelatin stab the growth becomes brownish. On agar slope with medium 335 to which 0.5 per cent of potassium or calcium nitrate had been added, the growth becomes opaque and iridescent. In all the standard media free from sugar the growth is scanty. In the presence of dextrose, saccharose, maltose, or glycerin, much more abundant growth takes place. Lactose and galactose increase the growth only slightly, while the addition of levulose tends to inhibit it. Two per cent of levulose added to media entirely inhibits the growth. Whether this is due to this sugar or to some impurity in it was not determined.

The organism does not produce any indol, hydrogen sulfite, or ammonia. It does not reduce nitrates and does not liquefy a 12-per-cent gelatin stab at 20° C., but when grown in milk it partly digests the casein without curdling the milk. It does not produce any gas from the sugars in fermentation tubes. From dextrose, maltose, and saccharose it produces a slight amount of some acid. Neutral litmus milk becomes alkaline after the organism has grown in it for about two weeks.

The organism of Canada field pea does not have any strikingly characteristic colony features by which it can be distinguished from other microorganisms. The group number of the organism, according to the chart of the Society of American Bacteriologists, was found to be B. 222.2322033.

PART II. INFLUENCE OF CERTAIN FACTORS ON NODULE PRODUCTION

The literature on the general subject of legume inoculation is very extensive; yet knowledge concerning the factors that influence nodule production is suprisingly meager. For the most part the investigations on this point are incidental and fragmentary. It has been shown by various investigators, however, that the nature of the medium in which the plant is grown has an influence on the production of nodules.

Rautenberg and Kühn (1864) grew *Vicia faba* in various nutrient solutions, and incidentally observed that in the nitrogen-free solution the beans developed an abundance of nodules, while in the solutions containing nitrogen no nodules were produced.

Hugo de Vries (1877) made a similar observation. He grew red clover in nutrient solutions, and the plants developed a large number of nodules in the nitrate-free solution but only a few or no nodules in the solution containing nitrates.

Vines (1888–1889) tested the influence of potassium nitrate on nodule development on *Vicia faba* grown in nutrient solutions and also in the soil. His experiments showed that potassium nitrate tends to inhibit nodule development, both in soil and water cultures.

Frank (1889) grew lupines and peas in humus soil and in humus-free soil. He found that the plants grown in the humus-free soil developed an abundance of nodules, and those grown in the soil rich in humus had no nodules. He offered the following explanation for this: "The lupines, and also the peas, obtain the same benefit from the nodule fungus as they do from the humus. Where humus is present, the plants prefer to obtain the nourishment from the humus and no nodules are developed; where humus is wanting, however, the nodule fungus infects the plants."[4]

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[4] Translation from the original German.

Hiltner (1900) showed that the addition of potassium nitrate to the nutrient solution in which legumes are grown has an injurious influence on nodule development, and he thinks this is due to the fact that the formation of bacteroids in the small nodules is hastened by the presence of the nitrate. He considers the bacteroids as degenerate and inactive forms of the nodule-forming organism.

A somewhat more extensive investigation of this subject was undertaken by Nobbe and Richter (1902). They attempted to determine the influence of potassium nitrate and of humous substances on the fixation of nitrogen by soy beans. They grew the plants in flowerpots in a rich soil and in a poor soil. For rich soil they used garden soil. The poor soil was prepared by mixing 4000 grams of sand and 2500 grams of garden soil. Potassium nitrate was added to the poor soil in the proportions of 500 and 1000 milligrams to 6500 grams of the soil. When the plants were harvested the total amount of dry substance and of nitrogenous matter was determined in each plant. The results of this experiment show that the function of the nodules for nitrogen fixation is injured to a high degree by the presence of potassium nitrate. The influence of humous substances is similar, but not so great.

The observation made by Frank, by Nobbe and Richter, and by others — namely, that soil rich in humous substances has a deleterious effect on nodule development — was confirmed also by Moore (1905). He grew soy beans in rich nitrogenous soil, in poor clay soil, and in poor sandy soil. Very few nodules developed on the soy beans grown in rich soil, while in the poor clay soil and in the poor sandy soil the plants developed an abundance of nodules.

The development of nodules may also be affected by other agencies. Gain (1893) attempted to determine the influence of moisture on nodule development on *Pisum sativum*, two varieties of *Lupinus albus*, and *Faba vulgaris*. He grew the plants in a field located in a region where rain was very scarce during the early part of the summer. One-half of the plat planted with each legume was watered artificially, and the other half was exposed to drought. Examination of the plants showed that the plants watered artificially had five and one-half times as many nodules as those not watered.

Marchal (1901) determined the influence of fifteen different nutritive mineral salts on nodule development on peas grown in Sachs' nutrient solution. He concluded from his experiments that nodule development is inhibited by the addition of the following nutrient salts in the given concentrations:

Alkaline nitrates, concentration 1 to 10,000
Ammonium salts, concentration 1 to 2,000
Potassium salts, concentration 1 to 300
Sodium salts, concentration 1 to 200

The influence of phosphates was variable, and calcium and magnesium salts stimulated nodule development. Marchal was of the opinion that the variation of the osmotic pressure, due to the presence of the different salts, may be the cause of this phenomenon.

Moore (1905) states that the addition of 1 per cent of sodium and potassium salts often entirely inhibits the formation of nodules, and smaller quantities considerably reduce their formation. The addition of calcium and magnesium salts, on the other hand, greatly favors nodule formation. Moore states further that this is not true with all the legumes, since the addition of calcium and magnesium carbonates is injurious to the formation of nodules on lupines and other plants adapted to acid soil.

In the following experiments the influence of various factors on nodule production has been investigated. The factors studied are light and darkness, aëration, moisture, various concentrations of nutrient solutions, and a considerable number of chemical substances.

EXPERIMENT 1

INFLUENCE OF AËRATION IN LIGHT AND IN DARKNESS

In this experiment Canada field peas were grown in an aqueous soil extract. The extract was prepared by taking one part of soil and four parts of water, by weight. The mixture was allowed to stand for two hours, and the liquid was then decanted and filtered. Twelve Erlenmeyer flasks of 300 cubic centimeters capacity were filled, and one pea was planted in each flask. Six of the flasks were covered with black paper and the other six were exposed to diffused light. Three flasks n each of the two series were aërated by passing a current of air through the liquid during the entire experiment. All the plants were inoculated with a pure culture of *Bacillus radicicola.* After twenty-four days the plants were examined for nodules.

Results

All the plants developed plenty of nodules. The inoculation with the pure culture had no apparent effect on the number of nodules. The soil extract was not sterilized and apparently had plenty of the organisms. The plants whose roots were kept in darkness had a greater abundance of nodules than those whose roots were exposed to light. The aëration as supplied in this experiment had no stimulative effect on either the number or the size of the nodules.

EXPERIMENT 2

INFLUENCE OF SOME NUTRIENT SOLUTIONS IN LIGHT AND IN DARKNESS

Ninety Erlenmeyer flasks of 300 cubic centimeters capacity were divided into five series, with eighteen flasks in each series. These flasks were filled with the following solutions, respectively: series 1, with medium 101; series 2, with Crone's solution; series 3, with Pfeffer's solution; series 4, with tap water; series 5, with soil extract (the same as was used n experiment 1). Six flasks from each series, three covered with black paper and three not covered, were inoculated with a pure culture of *Bacillus radicicola.* A second group of six flasks from each series were prepared in the same manner, but each flask was inoculated with 5 cubic centimeters of soil extract. A third group of six flasks from each series were prepared in the same manner but were not inoculated. One plant was grown in each flask. The water of transpiration was replaced each week. At the end of four weeks the plants were examined for nodules. The results are given in table 2.

Results

Not all the plants that were inoculated developed nodules. A few nodules developed on the plants grown in medium 101, in Crone's solution, and in Pfeffer's solution. In the soil extract all the plants developed nodules, although the plants grew better in Crone's solution and in Pfeffer's solution. In the tap water no nodules developed. More nodules developed on the roots kept in darkness than on those exposed to the light.

TABLE 2. INFLUENCE OF SOME NUTRIENT SOLUTIONS ON NODULE DEVELOPMENT

Culture solution*	Treatment	Exposure	Number of nodules		
			Plant 1	Plant 2	Plant 3
Medium 101......	Inoculated with *B. radicicola*	Light	None	None	None
		Dark	None	None	None
	Inoculated with soil extract.	Light	None	None	None
		Dark	None	None	Few
	Not inoculated............	Light	None	None	None
		Dark	None	None	None
Crone's solution...	Inoculated with *B. radicicola*	Light	None	None	None
		Dark	Few	Few	Few
	Inoculated with soil extract.	Light	None	None	None
		Dark	Few	Few	Few
	Not inoculated............	Light	None	None	None
		Dark	None	None	None
Pfeffer's solution..	Inoculated with *B. radicicola*	Light	None	None	None
		Dark	None	None	Few
	Inoculated with soil extract.	Light	None	Few	Few
		Dark	None	Few	Few
	Not inoculated............	Light	None	None	None
		Dark	None	None	None
Tap water........	Inoculated with *B. radicicola*	Light	None	None	None
		Dark	None	None	None
	Inoculated with soil extract.	Light	None	None	None
		Dark	None	None	None
	Not inoculated............	Light	None	None	None
		Dark	None	None	None
Soil extract.......	Inoculated with *B. radicicola*	Light	Present	Present	Present
		Dark	Present	Present	Present
	Not inoculated............	Light	Present	Present	Present
		Dark	Present	Present	Present

* See page 11.

EXPERIMENT 3

INFLUENCE OF POTASSIUM NITRATE IN LIGHT AND IN DARKNESS

In this experiment twelve glass cylinders, each of 5 liters capacity and 50 centimeters in height, were used. Six of these were filled with Crone's full nutrient solution, and the other six were filled with the same solution except that potassium chloride was substituted in place of potassium nitrate. Five plants were grown in each cylinder. The experiment was arranged in the following manner:

Series 1. Three of the cylinders filled with Crone's full nutrient solution were covered with black paper. Two of these were inoculated, and one was not inoculated.

Series 2. The other three cylinders filled with Crone's full nutrient solution were treated as was series 1, but were not covered with black paper.

Series 3. Three of the cylinders filled with Crone's solution in which potassium nitrate was replaced by potassium chloride, were covered with black paper. Two of these were inoculated, and one was not inoculated.

Series 4. The remaining three cylinders, with the same solution as was used for series 3, were treated as was series 3 but were not covered with black paper.

Results

When the plants were three weeks old, those grown in the solution with nitrate looked green and healthy, while those grown in the solution without nitrate were turning yellow and the lower leaves were dropping off. No difference in appearance was observed between the inoculated and the uninoculated plants. The uninoculated plants had no nodules; those grown in the presence of nitrate and inoculated had one or two nodules each; those grown in nitrate-free solution had about fifteen nodules each. Six weeks after planting, the plants grown in nitrate solution had thick, green leaves and thick roots, and no more nodules had developed on the inoculated plants. The plants grown in nitrate-free solution were yellowish except for the upper leaves, which were green; the roots were longer and more abundant than on the plants grown in nitrate solution. Nodules were abundant, continually developing on the new roots. The uninoculated plants in nitrate-free solution had no nodules and were practically dead.

The plants grown in nitrate-free solution with their roots exposed to light were slightly shorter than, and did not have quite as many nodules as, those grown in the same solution but with their roots kept in darkness. In the presence of the nitrate the development of certain green algæ interfered somewhat with root growth.

EXPERIMENT 4

INFLUENCE OF POTASSIUM NITRATE AND CALCIUM NITRATE IN PFEFFER'S SOLUTION

The procedure in this experiment was the same as in experiment 3. Only one plant was grown in each cylinder. Calcium nitrate was replaced by calcium chloride, and potassium nitrate was replaced by potassium chloride. The plants were kept until they began to blossom.

Results

In the solution with the nitrates, two or three nodules developed on each plant within twelve days after inoculation. No more nodules developed after that. In the cylinders not covered with black paper, algæ developed in abundance, and, surrounding the roots, dwarfed the plants. In the cylinders covered with black paper, also, some algæ developed in time, but they were far less abundant.

In the nitrate-free solution there was an abundant development of nodules. The nodules were more numerous on the plants grown in the cylinders covered with black paper than on those the roots of which were exposed to light. The root system of the plants grown in the nitrate-free solution was more developed than that of the plants grown in the nitrate solution.

An interesting point observed in this experiment and in experiment 3 was that the nodules developed, both in the nitrate solution and in the nitrate-free solution, immediately after inoculation. In the nitrate solution, however, no further development of nodules took place, while in the nitrate-free solution there was a continual development of new nodules on the new rootlets as time went on. This would seem to indicate either that the nodule-forming organisms were made inactive by the nature of the solution, or that the solution in some way affected the resisting power of the plants.

EXPERIMENT 5

INFLUENCE OF VARIOUS CONCENTRATIONS OF PFEFFER'S SOLUTION, BOTH WITH AND WITHOUT NITRATES

Wide-mouth bottles of 500 cubic centimeters capacity were used in this experiment. They were all covered with black paper. Two series were prepared, one with Pfeffer's full nutrient solution and the other with Pfeffer's solution in which the nitrates were replaced by the chlorides of the same metals. The following concentrations in each series were employed, taking the concentration of the full nutrient as 1: $\frac{1}{16}$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3, 4, and 8. Five plants were grown in each bottle, and all were inoculated. The duration of the experiment was three weeks.

Results

In the full nutrient solution a few nodules developed in the $\frac{1}{16}$ concentration. In the other concentrations no nodules appeared within the three weeks.

In the nitrate-free solution nodules developed best in concentration 1. In the $\frac{1}{2}$ and $\frac{1}{4}$ concentrations a few nodules appeared.

EXPERIMENT 6

INFLUENCE OF PFEFFER'S SOLUTION IN WHICH THE ESSENTIAL ELEMENTS WERE ABSENT

In this experiment the same vessels were used as in experiment 5. The solutions were prepared as follows:

$$\text{For solution minus nitrogen} \begin{cases} Ca(NO_3)_2 \text{ was replaced by } CaCl_2 \\ KNO_3 \text{ was replaced by } KCl \end{cases}$$

$$\text{For solution minus potassium} \begin{cases} KNO_3 \text{ was replaced by } NaNO_3 \\ KH_2PO_4 \text{ was replaced by } NaH_2PO_4 \\ KCl \text{ was replaced by } NaCl \end{cases}$$

For solution minus phosphorus, KH_2PO_4 was replaced by NaH_2PO_4
For solution minus sulfur, $MgSO_4$ was replaced by $MgCl_2$
For solution minus magnesium, $MgSO_4$ was replaced by $MgCl_2$
For solution minus iron, $FeCl_3$ was replaced by NaCl

One plant was grown in each bottle. The plants were examined three weeks after planting.

Results

Nodules developed only in the nitrate-free solution. The plants in most of the solutions in this experiment and in experiment 5 did not grow well and normally. It is possible that slightly different results might have been obtained had the length of time of the experiment been extended. It was observed, however, in these and in the other experiments, that the number and the size of the nodules on a plant are influenced by the rate and the amount of growth of the plant. In other words, any disturbing factor in the normal functions of a plant tends to hinder the development of nodules.

EXPERIMENT 7

INFLUENCE OF MOISTURE

In experiments 7, 8, and 9, the plants were grown in glass tumblers. In each tumbler was placed 300 grams of air-dry sandy soil containing less than 0.5 per cent of moisture. The following percentages of moisture were used: 5, 10, 15, 20, 25, 30, 40, 50, and 60, the percentage being based on the air-dry soil. Three tumblers were used for each percentage of moisture, and five plants were grown in each tumbler. The plants were kept in the greenhouse. They were watered every other day, the necessary amount of water to be added being determined by weighing. The soil is naturally well inoculated with the Canada field pea organism, but in addition to this each tumbler was inoculated with a pure culture of the organism. The duration of the experiment was four weeks.

Results

The best growth took place in 15, 20, and 25 per cent of moisture. In 5 and 10 per cent of moisture the plants grew very slowly, while in 50 and 60 per cent the roots rotted. Nodules were present on all the plants. The number of the nodules on each plant increased with the percentage of moisture up to 40 per cent. These results agree with those of Gain (1893), who found that a larger number of nodules develop when the plants are abundantly watered.

EXPERIMENT 8

INFLUENCE OF CERTAIN SUBSTANCES IN VARYING QUANTITIES

The same soil and the same kind of vessels were used in this experiment as in experiment 7. Three hundred grams of the air-dry soil was introduced into each glass tumbler; the substance to be tested was dissolved in the proper quantity of water, and this was added to each tumbler. Five plants were grown in each tumbler, and all the cultures were made in triplicate and were inoculated. The plants were allowed to grow for four weeks. The kind and the amount of the substance added, together with the results, are given in table 3:

TABLE 3. INFLUENCE ON NODULE DEVELOPMENT OF CERTAIN SUBSTANCES IN VARYING QUANTITIES

Substance used	Quantity added to 300 grams of soil (grams)	Condition of plants	Nodule development
KNO_3	0.25	Good growth	No nodules
	0.50	Poor growth	No nodules
	1.00	No growth	No nodules
	2.00	No growth	No nodules
$Ca(NO_3)_2$	0.25	Good growth	Few nodules
	0.50	Poor growth	No nodules
	1.00	No growth	No nodules
	2.00	No growth	No nodules
$MgSO_4$	0.25	Good growth	Nodules abundant
	0.50	Good growth	Nodules abundant
	1.00	Good growth	Nodules abundant
	2.00	Good growth	Nodules abundant
KH_2PO_4	0.25	Good growth	Nodules abundant
	0.50	Good growth	Nodules abundant
	1.00	Good growth	Nodules abundant
	2.00	Good growth	Nodules abundant
$CaCO_3$	0.25	Good growth	Nodules very abundant
	0.50	Good growth	Nodules very abundant
	1.00	Good growth	Nodules very abundant
	2.00	Good growth	Nodules very abundant
NH_4Cl	0.25	No growth	No nodules
	0.50	No growth	No nodules
	1.00	No growth	No nodules
	2.00	No growth	No nodules
$FeCl_3$	0.25	No growth	No nodules
	0.50	No growth	No nodules
	1.00	No growth	No nodules
	2.00	No growth	No nodules

TABLE 3 (*concluded*)

Substance used	Quantity added to 300 grams of soil (grams)	Condition of plants	Nodule development
Witte's peptone	0.25	Fair growth	Few nodules
	0.50	Poor growth	No nodules
	1.00	Poor growth	No nodules
	2.00	Poor growth	No nodules
Cane sugar	0.25	Good growth	Nodules present
	0.50	Good growth	Nodules present
	1.00	Good growth	Nodules present
	2.00	Good growth	Nodules present
	4.00	Poor growth	Nodules present
	8.00	Poor growth	No nodules
	16.00	Very poor growth	No nodules
Controls	Nothing added	Good growth	Nodules present

Results

The addition of $MgSO_4$, KH_2PO_4, and $CaCO_3$ in the concentrations used in the experiment had a beneficial effect on the development of nodules. Cane sugar at low concentrations had apparently no effect. At the concentrations of 4, 8, and 16 grams of sugar in 300 grams of soil, cane sugar was injurious, probably due to fermentation products and to stimulation of the development of microorganisms injurious to the plants and also to the development of nodules. The addition of NH_4Cl and $FeCl_3$ completely inhibited the growth of the plants. In the case of KNO_3 and $Ca(NO_3)_2$ the concentration of $\frac{1}{4}$ gram in 300 grams of soil had a beneficial effect on the growth of the plant, but an injurious effect on the development of nodules. A few nodules developed in the presence of $Ca(NO_3)_2$, but none in the presence of KNO_3. The higher concentrations of $Ca(NO_3)_2$ and KNO_3 inhibited nodule development and also caused injury to the plants.

EXPERIMENT 9

INFLUENCE OF CERTAIN ADDITIONAL SUBSTANCES

The method used in this experiment was the same as in experiment 8. Several additional chemicals were tested. In examining the plants, the number of nodules was counted and both the total number of nodules and the average per plant for each concentration of the substance were calculated. The results follow in table 4:

TABLE 4. Influence of Certain Additional Substances on Nodule Development

Chemical used	Quantity added to 300 grams of soil (grams)	Number of plants	Condition of plants	Number of small nodules	Number of large nodules	Total number of nodules	Number of nodules per plant
$Ca(NO_3)_2$	0.25	10	Small	6	......	6	0.6
	0.50	8	Small	......	......	0	0
	1.00	0	Plants killed	......	......	......	....
	2.00	0	Plants killed	......	......	......	....
Tannic acid	0.25	11	Good	40	138	178	16.2
	0.50	8	Good	50	41	91	11.4
	1.00	12	Good	17	50	67	5.6
	2.00	13	Good	100	20	120	9.2
KH_2PO_4	0.25	6	Good	10	50	60	10.0
	0.50	7	Good	50	58	108	15.4
	1.00	4	Small	30	10	40	10.0
	2.00	4	Small	16	5	21	5.3
$MgSO_4$	0.25	14	Good	46	100	146	10.4
	0.50	11	Good	26	50	76	6.9
	1.00	12	Good	60	41	101	8.4
	2.00	13	Good	24	32	56	4.3
KCl	0.25	7	Small	34	12	46	6.6
	0.50	0	Plants killed	......	......	......	....
	1.00	0	Plants killed	......	......	......	....
	2.00	0	Plants killed	......	......	......	....
KNO_3	0.25	14	Good	15	0	15	1.1
	0.50	14	Small	......	......	......	....
	1.00	3	Plants killed	......	......	......	....
	2.00	0	Plants killed	......	......	......	....
NH_4Cl	0.25	12	Good	0	0	0	0
	0.50	2	Small	0	0	0	0
	1.00	0	Plants killed	......	......	......	....
	2.00	0	Plants killed	......	......	......	....
Witte's peptone	0.25	11	Good	21	8	29	2.6
	0.50	11	Good	20	2	22	2.0
	1.00	9	Small	4	0	4	0.4
	2.00	1	Very small	......	......	......	....

TABLE 4 (*concluded*)

Chemical used	Quantity added to 300 grams of soil (grams)	Number of plants	Condition of plants	Number of small nodules	Number of large nodules	Total number of nodules	Number of nodules per plant
KOH	0.25	12	Good	35	50	85	7.1
	0.50	10	Good	10	45	55	5.5
	1.00	3	Small	11	25	36	12.0
	2.00	0	Plants killed	......	......	......	....
$Fe(NO_3)_3$	0.25	11	Good	4	0	4	0.4
	0.50	14	Good	1	0	1	0.1
	1.00	6	Small	0	0	0	0
	2.00	0	....	......	......	......	....
$Ca(H_2PO_4)_2$	0.25	8	Good	6	45	51	6.4
	0.50	14	Good	90	94	184	13.1
	1.00	12	Good	54	60	114	9.5
	2.00	5	Small	8	11	19	3.8
$CaSO_4$	0.25	11	Good	22	65	87	7.9
	0.50	10	Good	15	60	75	7.5
	1.00	14	Good	57	50	107	7.4
	2.00	10	Good	21	45	66	6.6
$FeCl_3$	0.25	0	Plants killed	......	......	......	....
	0.50	0	Plants killed	......	......	......	....
	1.00	0	Plants killed	......	......	......	....
	2.00	0	Plants killed	......	......	......	....
Starch	1.00	12	Good	40	82	122	10.2
	2.00	13	Good	59	40	99	7.6
	4.00	8	Good	18	42	60	7.5
Controls	....	18	Good	......	......	113	6.3
	....	14	Good	......	......	107	7.6

Results

The results of experiment 9 are similar to those of experiment 8. The following chemicals added to the soil at the concentrations used in the experiment were injurious to the plants and tended to inhibit the development of nodules: $Ca(NO_3)_2$, KCl, KNO_3, NH_4Cl, Witte's peptone, $Fe(NO_3)_3$, and $FeCl_3$. On the other hand, tannic acid, KH_2PO_4, $MgSO_4$, KOH, $Ca(H_2PO_4)_2$, $CaSO_4$, and starch exerted a beneficial influence on nodule development and appeared to have no injurious effect on the plants.

GENERAL DISCUSSION OF RESULTS OF EXPERIMENTS

The nine experiments reported in Part II, on the factors affecting the development of nodules on Canada field peas, are not extensive enough to allow any broad and general deductions, but the results point to several conclusions. Nodules develop readily on Canada field peas in nutrient solutions, provided the proper nutrient salts are added. Varying the concentration appears to have a marked influence on nodule development. Aëration has no appreciable effect. Nodules developed on the long roots at as great a depth as 30 centimeters below the surface of the nutrient solution. If air is essential for the development of nodules, enough of it was dissolved in the nutrient solution under the conditions in the experiments. The presence of nitrates in the nutrient solution or in the soil tends to inhibit the development of nodules; the reason for this is not known. If the plants are grown in the presence of nitrates for about a week and then inoculated, a few nodules will develop, but no further development of nodules takes place; in water cultures without nitrates and inoculated, a continuous nodule development takes place as long as the roots grow.

The chemical composition of the various soils used for agricultural purposes differs. What influence this has on the various groups of the nodule-forming organism and on nodule development has never been extensively investigated. The results of the foregoing experiments emphasize its importance. The limited distribution of the different groups of the nodule-forming organism in some soils, the failures in inoculations, and the difficulty in growing certain legumes, may be explained in certain cases as being due to the composition of the soil. It is known that the addition of lime to certain soils has a beneficial effect on nodule development and on the growth of some legumes. It is highly probable that the addition of other substances to the soil may be beneficial to other legumes.

PART III. INFLUENCE OF VARIOUS MEDIA ON THE INFECTING POWER AND THE VITALITY OF BACILLUS RADICICOLA

As indicated previously, and again further developed in the discussion of this subject, the view has been maintained that the infecting power, or "virulence," of the nodule-forming organism may be impaired by cultivating it on certain media. In order to determine whether or not such is the case, and also to determine the media most favorable for maintaining the vitality of the organism, the following experiments were made.

The term *virulence* has been used by previous investigators to mean the ability of the organism to penetrate the root and produce nodules. Since the term *virulence* in this connection, as also suggested by Edwards (in Marshall's Microbiologie), does not correctly apply to the legume bacteria, the term *infecting power* will be used throughout this paper.

Beyerinck (1890) was the first to isolate a pure culture of an organism from a nodule. Prazmowski (1890), Frank (1889), and Nobbe and others (1891), stimulated by Beyerinck's success, were also able to obtain pure cultures from the nodules of various legumes, and to produce nodules by inoculating the plants with the pure cultures. The experiments on the inoculation of legumes by pure cultures at once raised a question as to the classification of the nodule-forming organism, which question is intimately connected with the subject of the infecting power of the organism and the resistance of the plants. Do all the different organisms from the various species of legumes belong to several species, or do they belong only to one species but to several races or varieties? Can the organism from one species of legumes cause nodules on a different species of legumes? Is the relation between the legume and the organism a case of symbiosis or a case of parasitism? Does the organism have the biological or physiological character called *virulence* as understood by pathologists, and can this be altered or destroyed without injuring or destroying the other physiological activities? Do the host plants have a resistance in a pathological sense, and can this resistance be altered by the environmental factors without altering the morphology, the structure of the tissues, and the physiological activities of the plants? Is the resistance against the entrance of the organism into the root tissues different from the resistance against the development of the organism inside the root tissues? These and similar questions formed the foundation of the numerous investigations that were undertaken subsequently to the isolation of the pure culture of the nodule-forming organism, and nodule production by pure cultures.

Even before the isolation of the nodule-forming organism by Beyerinck, it was observed by Hellriegel (1886) that when peas, vetch, beans, clover, serradella, and lupines were inoculated with an infusion from the same soil, all the plants developed nodules except the serradella and the lupine. From this Hellriegel inferred that important differences must exist between the nodule bacteria of the different legumes.

Beyerinck (1888) was of the opinion that the nodule-forming organisms of the legumes belonged to one species, but that there were several groups and in each group a number of varieties. From the results of his subsequent investigation (1890) he was forced to change his former opinion. He considered the organism of Ornithopus and that of Vicia to be two distinct species.

Frank's investigations (1899) led him to believe that there was only one species among the nodule-forming organisms.

Salfeld (1888) grew peas and horse beans in "Hochmoorboden," and inoculated one part of them with sandy soil in which peas were grown and the other part with sandy soil in which lupines were grown. Both the peas and the horse beans inoculated with the pea soil developed nodules, while those inoculated with the lupine soil were free from nodules.

Laurent (1901) could produce nodules on dwarf peas by inoculating them with material from nodules of thirty different leguminous species, but he claimed that the number, size, and appearance of the nodules was influenced by the inoculating material of the different sources.

Kirchner (1896) grew about one hundred different species of legumes in the Hohenheimer botanical garden. He observed that all the different species of legumes developed nodules in the garden soil except the soy beans, although these had been grown in the garden for ten years. The soy beans did not produce nodules until they were inoculated with soil on which Japanese soy beans had been grown.

The investigations of Mazé (1898) led him to divide the nodule-forming organisms into two groups — those adapted to a neutral or an alkaline soil, and those adapted to an acid soil; the former infecting the plants that favor neutral and alkaline soil, and the latter infecting the plants that favor acid soil.

Nobbe, Schmid, Hiltner, and Hotter (1891) undertook a very extensive series of investigations on the general subject of nitrogen assimilation by leguminous plants. Much of the present information on this subject is due to these men, especially to Hiltner and Nobbe. They showed that the only way to study the relations between the nodule-forming organisms of the different legumes and the different species and varieties of legumes was to use pure cultures of the organisms for inoculation purposes, and not the soil infusions as was done by a number of previous investigators.

In a subsequent paper, Nobbe, Hiltner and Schmid (1895) arrived at the following important conclusions on the relation of nodule-forming organisms to the different species of legumes:

"The infecting power of the nodule bacteria of the various groups and species of legumes cannot be differentiated absolutely, but only in degree. The pure cultures from nodules of different species of legumes do not represent different species, but only different forms. We have not the least doubt that all the nodule bacteria of the different legumes we have studied, even those of Mimosæ, are one species, all belonging to *Bacillus radicicola* of Beyerinck. These bacteria, however, are influenced by the plants in whose roots they live to such a degree that their descendants are able to infect readily only that species of legumes to which the former host plant belonged, at the same time losing partly or completely the power to infect other species of legumes. When the legume is grown in a suitable soil, nodules will develop on the roots only when either those nodule bacteria are present which have lived previously on that legume species, or when the neutral nodule bacteria are present. The latter will be found in the soil where legumes have never been grown or where they have not been grown for a long time. If one legume is preponderantly grown in a soil, most of the neutral bacteria become influenced by this legume, and when a different legume is planted which is not closely related to the former no nodules will be formed, or only very few and faulty ones, and these will appear so late that they will be of very little value to the plants."[5]

By means of extensive experiments (Nobbe and Hiltner, 1896) it has been demonstrated that effective inoculation is obtained only when the plants are inoculated with bacteria from the nodules of the same species of legumes.

Moore (1905) conducted extensive cross-inoculation experiments, and maintains that "it is possible to cause the formation of nodules upon practically all legumes, no matter what was the source of the original organisms, provided they were cultivated for some time upon a synthetic nitrogen-free medium." He states further: "It is undoubtedly true that the long adaptation of the bacteria to the special conditions obtaining in a particular species of legume enables such organisms to produce more abundant nodules in a shorter length of time than bacteria isolated from some other legume and grown upon nitrogen-free media. While this is of considerable practical importance, and will probably always make it necessary to distribute the specific organism for the specific crop, it does not in any way indicate that the bacteria found in the nodules of beans, peas, clovers, etc., are separate species. The most that can be maintained is that there is a slight physiological difference due to the long association with a plant of a peculiar reaction which enables the bacteria more easily to penetrate the host upon which they have been accustomed to grow. These slight racial characteristics can readily be broken down by cultivation in the laboratory, and it is entirely possible to secure a universal organism capable of producing a limited number of nodules upon all the legumes which now possess these growths."

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[5] Translation from the original German.

Hopkins (1904) found that the organism from sweet clover readily inoculates alfalfa.

Nobbe and Hiltner (1900) undertook to train the nodule-forming organism of peas and that of beans so that the former may cause nodules on beans and the latter on peas. They succeeded in doing this, and drew the following conclusions:

1. The nodule-forming organism from peas can be trained to produce nodules on beans, and that from beans to produce nodules on peas.
2. Although some nodules are produced in both cases, the organisms do not assimilate any nitrogen at first.
3. If the pea organism that caused nodules on beans is isolated and beans are inoculated with it a second time, the organism then infects the beans more readily than at the first inoculation and its power to assimilate nitrogen is increased. The organism of beans behaves in the same manner when made to infect peas.

Kellerman (1912) reports that Mr. Leonard has succeeded in securing abundant inoculation on soy beans, lupines, and alfalfa from an organism of a culture originally isolated from the alfalfa nodule and kept on an artificial medium in the laboratory for about six years. Kellerman, therefore, is of the opinion that the nodule-forming organisms of all the Leguminosæ should be considered as a single species.

The evidence from the investigations mentioned above points to two conclusions: (1) that, with some exceptions, the nodule-forming organism from one legume does not cause nodules on another legume; (2) that the organism from one legume may be trained to cause nodules on any other legume. The evidence for the latter conclusion, however, is not final.

About 1895 a German company placed nitragin on the market — a pure culture of the nodule-forming organism for inoculation purposes. The cultures were propagated on gelatin and their preparation was based on the results of the investigations of Nobbe and Hiltner. These cultures were extensively tested both in Germany and in other countries, and, as judged by the reports of these tests, the cultures proved only partially successful. As a result of these adverse reports on nitragin, Nobbe and Hiltner (1899 a) undertook to ascertain the cause of the low efficiency of their cultures. They had already shown that the nodule-forming organism can be trained to infect other legume species than that of its host plant, when they trained the organism from peas to produce nodules on beans and that from beans to cause nodules on peas. They went a step further and demonstrated that the infecting power of the organisms can be altered in degree. They stated that the propagation of the organism on artificial media increases or diminishes the vitality, and that in general nitrogenous media are injurious to the vitality of the organism.

Moore (1905) also reports that as a result of numerous trials it has been found that, although the bacteria increase most rapidly on a medium rich in nitrogen, the resulting growth is usually of very much reduced vitality, and when put into the soil these organisms have lost the ability to break up into the minute forms necessary in order to penetrate the root hairs.

In a further study of this subject, Hiltner (1900) was led to believe that this variableness in the infecting power of the nodule-forming organism is the determining factor of the number and size of the nodules on every plant when grown under otherwise favorable conditions. He took some older plants that already had nodules on their roots, and placed them in a nutrient solution without any nitrogen. Repeated inoculation with its own organism did not produce any nodules on the new rootlets. When fall came, and the leaves began to turn yellow and drop, and the organisms in the nodules became weaker than those in the solution, nodules began to develop on the rootlets. When Hiltner took older plants that had no nodules on their roots and placed them in a similar solution to that used with the other plants, an immediate development of nodules took place on the new rootlets. From this and other experiments, Hiltner concluded that " the active nodules produce immunity in the plant against the bacteria that possess the same or a lower degree of infecting power than those already living in the nodules of that plant. Only bacteria of higher infecting power are able to enter the root tissue."[6]

Süchting (1904), believing that Hiltner's theory of the infecting power of the organism and its relation to nodule development was not sound, undertook a series of interesting experiments on this subject, as well as an elaborate discussion of Hiltner's theory and of his own theory. In his experiments Süchting attempted to ascertain three points: (1) Have the organisms that produce the first nodules on the plant less infecting power than those that produce nodules on the same plant subsequently? (2) Does the symbiosis with the plant influence the infecting power of the organism? (3) Does the artificial medium influence the infecting power of the organism?

From his experiments Süchting drew the following conclusions:

1. The infecting power of the bacteria is not proportional to the age of the nodule.

2. The passage of the bacteria through the host plant does increase their infecting power. Their infecting power does not vary at the different stages of the plant's vegetative period, and the feeding of the plant by potassium nitrate is injurious to the bacteria in the nodules.

3. When propagated on artificial media the lupine bacteria lose their infecting power on some media and may exist in a so-called pseudo form. On neutral media the bacteria retain their infecting power better than on acid media. The horse-bean bacteria do not behave in the same manner, but keep their infecting power for a long time on suitable media.

Lewis and Nicholson (1905), on the other hand, found by their experiments that " It seems that the presence or absence of nitrogen in the culture media is not the determining factor in maintaining the activity of the germ. Cultivation in the presence of the amount of nitrogen usually present in bouillon with from two to five per cent of cane sugar or glucose, preferably the former, has given best results in a l of the work connected with the experiment."

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[6] Translation from the original German.

In the following pages data are presented on experiments conducted through several years in an attempt to alter the "virulence"— that is, the infecting power — of the organism. In experiments 10, 12, and 13, *Bacillus radicicola* of Canada field pea was used; in experiment 11 that of alfalfa was used. The organisms were propagated and kept on various media. Their infecting power was tested and measured by the nodule development in plants grown in a sterilized sandy soil.

EXPERIMENT 10

INFLUENCE OF CLAY, LOAM, SAND, AND CARBORUNDUM

In this experiment the organism was grown on clay, sandy loam, sandy soil, fine quartz sand, coarse quartz sand, and carborundum. One hundred grams of each substance, air-dried, was introduced into Erlenmeyer flasks of 300 cubic centimeters capacity. After sterilization the media were heavily seeded with *B. radicicola.* This was accomplished by introducing into each flask the growth of *B. radicicola* from one agar slope, along with the necessary quantity of water. The amount of moisture added to each medium was about five per cent less than its capacity for holding water.

Two series of flasks were prepared. In series 1 the media, as soon as seeded with the organism, were spread on sterile paper and allowed to dry at room temperature. The time required for their complete drying was about six hours. In series 2 the media were left in the flasks, plugged with cotton, and allowed to stand in the laboratory. Drying of the media in this series was very gradual. The infecting power of the organism in these cultures was tested by inoculating plants. For this purpose Canada field peas were grown in sterilized soil in flowerpots, and were inoculated with the respective cultures at the time of seeding. Inoculation was accomplished by scattering one gram of the inoculating material over the soil in the flowerpots. The first test was made when the cultures were ten days old and the second test when the cultures were forty-six days old. When the plants were three weeks old they were examined for presence of nodules. The results are presented in tables 5 and 6:

TABLE 5. Results of First Inoculation Test. The Cultures Were Ten Days Old

	Plants inoculated with	Number of plants	Number of plants with nodules	Total number of nodules	Number of nodules per plant
Series 1	Clay	17	4	31	1.8
	Sandy loam	23	13	81	3.5
	Sandy soil	14	7	45	3.2
	Fine quartz sand	15	3	9	0.6
	Coarse quartz sand	19	0	0	0
	Carborundum	11	2	25	2.3
Series 2	Clay	10	6	51	5.1
	Sandy loam	15	10	90	6.0
	Sandy soil	18	10	67	3.7
	Fine quartz sand	13	7	92	7.1
	Coarse quartz sand	18	12	94	5.2
	Carborundum	14	4	23	1.6
	Agar slope culture	19	15	90	4.7
	Controls	13	3	15	1.2

TABLE 6. Results of Second Inoculation Test. The Cultures Were Forty-six Days Old

	Plants inoculated with	Number of plants	Number of plants with nodules	Total number of nodules	Number of nodules per plant
Series 1	Clay	5	5	53	10.6
	Sandy loam	12	11	79	6.6
	Sandy soil	12	10	151	12.6
	Fine quartz sand	10	10	58	5.8
	Coarse quartz sand	4	4	41	10.3
	Carborundum	11	7	113	10.3
Series 2	Clay	3	3	20	6.7
	Sandy loam	4	3	45	11.3
	Sandy soil	14	14	188	13.4
	Fine quartz sand	8	7	190	23.8
	Coarse quartz sand	7	6	78	11.1
	Carborundum	10	4	32	3.2
	Controls	36	13	120	3.3

Results

In both tests there was a certain amount of infection due to other sources than the inoculating materials. In the first test three of the thirteen plants used as controls developed nodules, while in the second test thirteen of the thirty-six control plants developed nodules. It was noticed, however, that, as a rule, if contamination took place subsequently to the inoculation and the plants were examined within four weeks after planting, the nodules due to the contamination were small and developed on the lateral roots near the surface of the soil, whereas nodules resulting from inoculation always appeared first on the taproot and were larger. Nevertheless, the results as shown in the two tables do not allow any clear-cut deductions. The plants of the first test (table 5) were examined three weeks after planting; the plants of the second test (table 6) were kept for four weeks, which probably accounts for the larger number of nodules on those plants.

It appears certain that *B. radicicola* remained alive and retained its infecting power in practically all the substances for forty-six days. Carborundum gave the poorest results. The plants inoculated with this substance developed only small nodules, mostly on the side roots — a fact pointing to subsequent infection. As regards the two series, much better inoculation was obtained from series 2 in both tests. In the first test 99 plants were inoculated with the cultures of series 1. These plants produced 191 nodules, which is an average of 1.9 nodules per plant. The 88 plants inoculated with the cultures from series 2 produced 417 nodules, which is an average of 4.7 nodules per plant. From similar calculations for the second test, it is found that the average number of nodules per plant was 9.2 in series 1, and 12 in series 2. In both tests the plants inoculated with the cultures of series 2 produced more nodules than those inoculated with the cultures of series 1. The drying of the substances in series 1 either reduced the infecting power of *B. radicicola*, or reduced the number of the organisms, or had both results.

EXPERIMENT 11

INFLUENCE OF HYDROCHLORIC ACID, SODIUM HYDROXIDE, AND CANE SUGAR, IN VARYING CONCENTRATIONS

In this experiment *B. radicicola* of alfalfa was isolated and identified according to the procedure described in Part I of this paper. The organism was propagated on media 334, 335, and 337 (page 11), to which were added various amounts of hydrochloric acid (HCl) and sodium hydroxide (NaOH). Cane sugar was added in various amounts to medium 334.

Ten cubic centimeters of the media were introduced into each test tube and sterilized. While the agar was still melted the various additions were made to the tubes, and, after thorough mixing, the tubes were sloped.

All cultures were made in duplicate. At the end of three weeks stain preparations were made from each slope for morphological study, carbol fuchsin being used for staining. The media employed and the results of the morphological study are given in table 7:

TABLE 7. MORPHOLOGICAL VARIATION OF B. RADICICOLA ON THE DIFFERENT MEDIA

Medium	Morphological appearance	Multiplication
Medium 334, 10 cc.		
+ 0	Small, short cells	Fair
+ 0.1 cc. N/1 HCl	Short rods, slightly stained	Fair
+ 0.5 cc. N/1 HCl	Short rods, slightly stained	Poor
+ 1.0 cc. N/1 HCl	Short rods, slightly stained	None
+ 1.5 cc. N/1 HCl	Short rods, slightly stained	None
+ 2.0 cc. N/1 HCl	Short rods, slightly stained	None
+ 3.0 cc. N/1 HCl	Short rods, slightly stained	None
+ 0.1 cc. N/1 NaOH	Short rods, well stained	Good
+ 0.5 cc. N/1 NaOH	Short rods, well stained	Good
+ 1.0 cc. N/1 NaOH	Short rods, well stained	Poor
+ 1.5 cc. N/1 NaOH	Short rods, well stained	Poor
+ 2.0 cc. N/1 NaOH	Short rods, well stained, few small bacteroids	Very poor
+ 3.0 cc. N/1 NaOH	Short rods, well stained, few small bacteroids	Doubtful
+ 0.1 per cent cane sugar	Cells vary in size, bacteroids present	Good
+ 0.5 per cent cane sugar	Cells vary in size, bacteroids present	Good
+ 1.0 per cent cane sugar	Cells vary in size, bacteroids present	Good
+ 1.5 per cent cane sugar	Cells vary in size, bacteroids present	Good
+ 2.0 per cent cane sugar	Cells vary in size, bacteroids present	Good
+ 3.0 per cent cane sugar	Cells vary in size, bacteroids present	Good
+ 4.0 per cent cane sugar	Cells vary in size, bacteroids more abundant	Good
+ 5.0 per cent cane sugar	Cells vary in size, bacteroids more abundant	Good
+ 6.0 per cent cane sugar	Cells vary in size, bacteroids more abundant	Fair
+ 8.0 per cent cane sugar	Cells vary in size, bacteroids very abundant	Fair
+ 10.0 per cent cane sugar	Cells vary in size, bacteroids very abundant	Poor

TABLE 7 (*concluded*)

Medium	Morphological appearance	Multiplication
Medium 335, 10 cc.		
+ 0	Variation in size and shape, few bacteroids	Good
+ 0.1 cc. N/1 HCl	Variation in size and shape, few bacteroids	Good
+ 0.5 cc. N/1 HCl	Very few rods	Poor
+ 1.0 cc. N/1 HCl	No organisms visible	Doubtful
+ 1.5 cc. N/1 HCl	No organisms visible	None
+ 2.0 cc. N/1 HCl	No organisms visible	None
+ 3.0 cc. N/1 HCl	No organisms visible	None
+ 0.1 cc. N/1 NaOH	Variation in size and shape, few bacteroids	Very good
+ 0.5 cc. N/1 NaOH	Variation in size and shape, few bacteroids	Very good
+ 1.0 cc. N/1 NaOH	Greater variation, more bacteroids than in 0.5	Good
+ 1.5 cc. N/1 NaOH	Greater variation, more bacteroids than in 0.5	Good
+ 2.0 cc. N/1 NaOH	Greater variation, more bacteroids than in 0.5	Poor
+ 3.0 cc. N/1 NaOH	Cells stained deeper than others, bacteroids present	Doubtful
Medium 337, 10 cc.		
+ 0	Pronounced variation in shape and size of cells.	Good
+ 0.1 cc. N/1 HCl	Small cells, irregular shape and size	Good
+ 0.5 cc. N/1 HCl	Very slender cells, irregular shape and size	Poor
+ 1.0 cc. N/1 HCl	Extremely small cells	Very poor
+ 1.5 cc. N/1 HCl	Extremely small cells	Doubtful
+ 2.0 cc. N/1 HCl	Extremely small cells	None
+ 3.0 cc. N/1 HCl	No organisms visible	None
+ 0.1 cc. N/1 NaOH	Short, irregular cells	Abundant
+ 0.5 cc. N/1 NaOH	Short, irregular cells	Abundant
+ 1.0 cc. N/1 NaOH	Shape and size extremely varied, many bacteroids	Abundant
+ 1.5 cc. N/1 NaOH	Large cells, varying in shape and size, many bacteroids	Good
+ 2.0 cc. N/1 NaOH	Large cells, varying in shape and size, many bacteroids	Good
+ 3.0 cc. N/1 NaOH	Very large cells, varying in shape and size, many bacteroids	Poor

In order to test the infecting power of the different cultures, three flowerpots of alfalfa plants grown in sterile soil were inoculated with the organism from each test tube. Four weeks after inoculation the plants were examined for nodule development. The results of the inoculations are presented in table 8. Fifty-eight plants in flowerpots were used as controls. These plants were grown in sterile soil and were not inoculated. The control plants were distributed among the other plants in order to see to what extent an infection from neighboring flower-pots may take place.

TABLE 8. INFECTING POWER OF VARIOUS CULTURES. B. RADICICOLA WAS PROPAGATED FOR THREE WEEKS ON THE DIFFERENT MEDIA

Medium	Number of plants inoculated	Total number of nodules	Number of nodules per plant
Medium 334, 10 cc.			
+ 0	42	265	6.3
+ 0.1 cc. N/1 HCl	50	320	6.4
+ 0.5 cc. N/1 HCl	43	188	4.3
+ 1.0 cc. N/1 HCl	32	0	0
+ 1.5 cc. N/1 HCl	38	6	0.2
+ 2.0 cc. N/1 HCl	53	22	0.4
+ 3.0 cc. N/1 HCl	35	7	0.2
+ 0	37	184	5.0
+ 0.1 cc. N/1 NaOH	43	275	6.4
+ 0.5 cc. N/1 NaOH	48	234	4.9
+ 1.0 cc. N/1 NaOH	47	304	6.5
+ 1.5 cc. N/1 NaOH	34	168	4.9
+ 2.0 cc. N/1 NaOH	27	94	3.5
+ 3.0 cc. N/1 NaOH	27	75	2.8
+ 0.1 per cent cane sugar	46	182	4.0
+ 0.5 per cent cane sugar	35	124	3.5
+ 1.0 per cent cane sugar	32	117	3.7
+ 1.5 per cent cane sugar	48	198	4.1
+ 2.0 per cent cane sugar	38	140	3.7
+ 3.0 per cent cane sugar	29	125	4.3
+ 4.0 per cent cane sugar	35	138	3.9
+ 6.0 per cent cane sugar	33	132	4.0
+ 8.0 per cent cane sugar	33	110	3.3
+ 10.0 per cent cane sugar	30	85	2.8
Medium 335, 10 cc.			
+ 0	29	132	4.6
+ 0.1 cc. N/1 HCl	34	183	5.4
+ 0.5 cc. N/1 HCl	50	6	0.1
+ 1.0 cc. N/1 HCl	68	0	0
+ 1.5 cc. N/1 HCl	50	22	0.4
+ 2.0 cc. N/1 HCl	56	5	0.1
+ 3.0 cc. N/1 HCl	58	4	0.1
+ 0.1 cc. N/1 NaOH	33	186	5.6
+ 0.5 cc. N/1 NaOH	32	131	4.1
+ 1.0 cc. N/1 NaOH	30	212	7.1
+ 1.5 cc. N/1 NaOH	36	284	7.9
+ 2.0 cc. N/1 NaOH	30	180	6.0
+ 3.0 cc. N/1 NaOH	21	109	5.2

TABLE 8 (*concluded*)

Medium	Number of plants inoculated	Total number of nodules	Number of nodules per plant
Medium 337, 10 cc.			
+ 0	23	54	2.3
+ 0.1 cc. N/1 HCl	32	50	1.6
+ 0.5 cc. N/1 HCl	37	110	3.0
+ 1.0 cc. N/1 HCl	36	77	2.1
+ 1.5 cc. N/1 HCl	36	23	0.6
+ 2.0 cc. N/1 HCl	33	94	2.8
+ 3.0 cc. N/1 HCl	32	132	4.1
+ 0.1 cc. N/1 NaOH	26	80	3.1
+ 0.5 cc. N/1 NaOH	27	120	4.4
+ 1.0 cc. N/1 NaOH	39	174	4.5
+ 1.5 cc. N/1 NaOH	31	114	3.7
+ 2.0 cc. N/1 NaOH	32	154	4.8
+ 3.0 cc. N/1 NaOH	33	87	2.6
Controls	735	*624	0.8

* Nodules were present in only thirteen out of fifty-eight pots

Results

The extent of multiplication and the description of the morphological characters of the organisms in the various media are summarized in table 7. No visible increase in the number of organisms was found when 1 cubic centimeter or more of the normal solution of HCl was added to 10 cubic centimeters of each medium. The few organisms found on these slopes were probably those that were introduced when the slopes were inoculated. In medium 337 slight multiplication took place on slopes of 10 cubic centimeters of the medium plus 1 cubic centimeter of the normal solution of HCl. The best multiplication occurred in media 335 and 337 when 0.1 to 1 cubic centimeter of the normal solution of NaOH was added to 10 cubic centimeters of the medium. This was particularly noticeable in medium 337. The addition of 3 cubic centimeters of the normal solution of NaOH to 10 cubic centimeters of the medium practically inhibited multiplication. The bacteroids developed more readily when NaOH was added.

The addition of sugar to medium 334, up to 10 per cent, caused more rapid multiplication and also the development of bacteroids.

In medium 334 multiplication of the organisms was very slow and only a few bacteroids developed in the three weeks. In medium 337 multiplication was abundant and bacteroids developed.

The results of inoculation are given in table 8. There were two hundred and eight flowerpots, which were crowded together because of lack of space. Fifty-eight flowerpots contained control plants, which were scattered among the inoculated pots. The plants in forty-five of the control pots developed no nodules; thirteen of the control pots were contaminated, and these were located mostly among the flowerpots inoculated with the organisms grown on medium 337. This contamination occurred in spite of the precautions taken to prevent the organisms from being carried from one flowerpot to another when the plants were watered. In these experiments and in others not reported in this paper, it was found that when a large number of flowerpots were used at one time it was difficult to prevent infection from other sources than that of the inoculating material. This was particularly true in the case of those legumes that produce an abundance of nodules in the soil of this region, and when the plants were allowed to grow for longer periods than three weeks. The results in this experiment are marred by a certain amount of contamination. The data in table 8 are so arranged as to show the total number of nodules on all the plants inoculated with the same material, and also to show the average number of nodules to each of these plants. Basing the conclusions on the mere number of nodules overemphasizes the importance of the contamination. When the plants were examined, and the size, location, and evenness of distribution of the nodules among the plants in the same flowerpot were noted, in addition to their total number, much more reliable evidence was obtained. In view of this, the following conclusions seemed to be warranted:

When *B. radicicola* of alfalfa is propagated on media 334, 335, and 337 and kept for three weeks, multiplication of the organism takes place and the infecting power is not lost. A slight reduction in infecting power seems to be apparent on medium 337. The addition of 0.1 cubic centimeter of the normal solution of HCl to 10 cubic centimeters of each of these media very slightly diminished the amount of growth, but the infecting power was not affected. When 0.5 cubic centimeter of the acid solution was added, the amount of growth was decidedly reduced on media 334 and 335 and the infecting power also was reduced. The influence of this amount of acid in medium 337 was not so pronounced. The addition of 1 cubic centimeter or more of the acid solution completely inhibited the growth and the infecting power of the organism on all three media.

The addition of 0.1, 0.5, 1, 1.5, and 2 cubic centimeters, respectively, of the normal solution of NaOH, to 10 cubic centimeters of each of the media, increased the amount of growth and also the effectiveness of the inoculation. The addition of 3 cubic centimeters of the normal solution of NaOH reduced the amount of growth and the infecting power. The heaviest growth took place on medium 337 to which the above amounts of NaOH were added, but the plants inoculated with these cultures did not produce as many nodules as did the plants inoculated with the organisms propagated on media 334 and 335 — a fact that suggests the possibility of a reduction in infecting power.

The addition of cane sugar to medium 334 in the amounts indicated in table 8 has a beneficial influence on the multiplication of the organism. The infecting power does not seem to be affected by it. All the cultures in which any multiplication was observed produced positive inoculation, so that the infecting power of the organism was not destroyed. The variations in the number of nodules on the plants inoculated by the different cultures seem to indicate that the organisms propagated on medium 337 partly lost their infecting power. This measure of infecting power, however, is not accurate, and other explanations might easily be supplied. The most noticeable point was that positive inoculation was produced by all the cultures in which any multiplication took place, so that the growth and the infecting power seem to run parallel.

EXPERIMENT 12

INFLUENCE OF SOME OTHER MEDIA

The organism, isolated as described in Part I, was kept in the laboratory for two years on agar slopes, medium 335, before this experiment was started. During this time the organism was continually exposed to diffused light and to the ordinary variations in temperature and humidity. The organism was then propagated on agar slopes, medium 334, to which various substances were added as is indicated in tables 9 and 10. Twelve

TABLE 9. DESCRIPTION OF THREE-WEEKS-OLD GROWTH OF B. RADICICOLA ON AGAR SLOPES

Medium 334 with	Description of growth
2.0 per cent cane sugar	Good, watery
2.0 per cent dextrose	Good, watery
2.0 per cent levulose	Not visible
2.0 per cent lactose	Not so good as with cane sugar
2.0 per cent galactose	Good, watery
10.0 per cent cane sugar	Good, watery
20.0 per cent cane sugar	Good, watery
40.0 per cent cane sugar	Slight, very transparent
2.0 per cent glycerin	Good, watery
2.0 per cent mannite	Good, watery
0.1 per cent asparagin	Fair
1.0 per cent salicin	Fair, whitish, not watery
0.5 per cent amygdalin	Fair, whitish
0.5 per cent resorcin	Hardly visible
0.2 per cent phloroglucin	Not visible
0.5 per cent potassium oxalate	Not visible
0.5 per cent potassium citrate	Not visible
0.1 per cent potassium nitrate	Poor, fluorescent, opaque
0.2 per cent potassium nitrate	Poor, fluorescent, opaque
0.6 per cent potassium nitrate	Poor, fluorescent, opaque
0.1 per cent calcium nitrate	Medium, very opaque, fluorescent, tough, brownish
0.2 per cent calcium nitrate	Medium, very opaque, fluorescent, tough, brownish
0.6 per cent calcium nitrate	Medium, very opaque, fluorescent, tough, brownish
1.0 per cent gelatin	Medium, juicy, opaque
5.0 per cent gelatin	Poor
1.0 per cent Witte's peptone	Abundant, juicy
2.0 per cent Witte's peptone	Abundant, juicy
5.0 per cent Witte's peptone	Not visible
0.2 per cent Merck's peptone	Abundant, fluorescent, whitish to brownish
1.0 per cent Merck's peptone	Abundant, fluorescent, whitish to brownish
3.0 per cent Merck's peptone	Not visible

tubes of each medium were prepared, and after being sterilized they were sloped and inoculated.

The following substances were used alone as media: soy bean hay, ground; soy bean roots, ground; Canada field pea hay, ground; Canada field pea roots, ground; Canada field pea seeds, ground; compost (well-decomposed cow feces), ground; partly decomposed cow feces, ground; fresh cow feces, ground; corn meal; sawdust; wheat bran; wheat middlings; sandy soil; muck; cornstarch. These substances were dried at 100° C., ground fine, and then introduced into test tubes 25 x 180 millimeters in size. Each tube was filled to one-third its capacity. Twelve test tubes were prepared for each medium, plugged with cotton, and sterilized in the autoclave for one hour at 15° C. For inoculation 10 cubic centimeters of sterile water in which the organisms were suspended was added to each test tube. At the end of two weeks the test tubes were sealed with paraffin in order to reduce evaporation. All the cultures were kept in the laboratory at room temperature.

Three tests were made in order to determine the infecting power of the organism propagated on the various media. The first test was made at the end of the third week, the second at the end of the tenth week, and the third at the end of the twentieth week. In these tests one test tube was taken from each of the media, the total number of organisms in each of these tubes was determined by the plate method, and Canada field pea plants were inoculated.

The plants were grown in sterilized sandy soil in flowerpots, three flowerpots being inoculated with each culture. When the plants were three weeks old they were examined and the nodules on each plant were counted. In table 9 is given a description of the three-weeks-old growth

TABLE 10. Number of Organisms in the Various Media at the Time the Tests Were Made

Medium	Number of organisms per gram		
	Cultures three weeks old	Cultures ten weeks old	Cultures twenty weeks old
Soy bean hay	0	0	0
Soy bean roots	0	0	0
Canada field pea hay	Few	0	0
Canada field pea roots	1,500,000,000	306,000,000	0
Canada field pea seeds	10,000,000,000	185,000,000	....
Compost	340,000,000	75,000,000	120,000,000
Partly decomposed cow feces	120,000,000	75,000,000	3,240,000,000
Fresh cow feces	60,000,000	440,000,000	810,000,000
Corn meal	7,200,000,000	Contamination	97,000,000
Sawdust	32,000,000	3,000,000	3,500,000
Wheat bran	10,000,000,000	370,000,000	600,000,000
Wheat middlings	4,000,000,000	390,000,000	420,000,000
Sandy soil	60,000,000	37,000,000	20,000,000
Muck	620,000,000	222,000,000	240,000,000
Cornstarch	200,000,000	55,000,000	97,000,000

TABLE 10 (*concluded*)

Medium 334 with	Number of organisms on one agar slope		
	Cultures three weeks old	Cultures ten weeks old	Cultures twenty weeks old
2.0 per cent cane sugar	No count made	300,000,000	90,000,000
10.0 per cent cane sugar	No count made	3,000,000	5,000,000
20.0 per cent cane sugar	No count made	1,000,000	10,000
40.0 per cent cane sugar	No count made	16,000,000	No colonies
2.0 per cent dextrose	No count made	31,000,000	52,000,000
2.0 per cent levulose	No count made	400,000	No colonies
2.0 per cent lactose	No count made	Plates spoiled	180,000
2.0 per cent galactose	No count made	3,000,000	600,000
2.0 per cent glycerin	No count made	250,000,000	20,000,000
2.0 per cent mannite	No count made	....	25,000,000
0.1 per cent asparagin	No count made	300,000,000	108,000,000
1.0 per cent salicin	No count made	No colonies	No colonies
0.5 per cent amygdalin	No count made	250,000,000	No colonies
0.5 per cent resorcin	No count made	No colonies	No colonies
0.2 per cent phloroglucin	No count made	Few	No colonies
0.1 per cent potassium nitrate	No count made	120,000,000	72,000,000
0.2 per cent potassium nitrate	No count made	74,000,000	224,000,000
0.6 per cent potassium nitrate	No count made	259,000,000	144,000,000
0.1 per cent calcium nitrate	No count made	280,000,000	486,000,000
0.2 per cent calcium nitrate	No count made	150,000,000	108,000,000
0.6 per cent calcium nitrate	No count made	400,000,000	130,000,000
1.0 per cent gelatin	No count made	150,000,000	64,000,000
5.0 per cent gelatin	No count made	50,000,000	Plates spoiled
1.0 per cent Witte's peptone	No count made	50,000	216,000,000
2.0 per cent Witte's peptone	No count made	Contaminated	648,000,000
5.0 per cent Witte's peptone	No count made	No colonies	No colonies
0.2 per cent Merck's peptone	No count made	22,000,000	8,000,000
1.0 per cent Merck's peptone	No count made	300,000,000	324,000,000
3.0 per cent Merck's peptone	No count made	No colonies	No colonies
0.5 per cent potassium oxalate	No count made	No colonies	No colonies
0.5 per cent potassium citrate	No count made	No colonies	No colonies

of *B. radicicola* on the agar slopes to which the various chemicals were added. The number of organisms in the cultures on the various media at the time they were tested for their efficiency to produce nodules is given in table 10. The results of the inoculations are shown in tables 11, 12, and 13.

Results

Growth and number of organisms (*tables 9 and 10*)

Bacillus radicicola of Canada field pea produces scant growth on agar slope medium 334. Some of the substances that were added to this medium retarded or completely inhibited the growth; others had no appreciable effect on the growth; and still others caused a decided increase in the growth as compared with that on medium 334 alone. (Table 9.) No visible growth was produced when the following substances were added: levulose 2 per cent, phloroglucin 0.2 per cent, potassium oxalate 0.5 per cent, potassium citrate 0.5 per cent, Witte's peptone 5 per cent, Merck's peptone 3 per cent. The growth in the remaining cultures was watery, almost transparent, very viscous, especially in the presence of sugars.

The number of organisms on the agar slopes was determined when the cultures were ten and twenty weeks old, and in the case of the substances in which the organism was propagated the determination was made at three, ten, and twenty weeks. The data in table 10 show a wide variation; but in general, wherever a visible amount of growth appeared on the agar slopes large numbers of organisms were found. At the end of ten weeks no organisms were found in the presence of salicin 1 per cent, resorcin 0.5 per cent, Witte's peptone 5 per cent, Merck's peptone 3 per cent, potassium oxalate 0.5 per cent, and potassium citrate 0.5 per cent. At the end of twenty weeks, in addition to the above no organisms were found in the presence of cane sugar 40 per cent, levulose 2 per cent, amygdalin 0.5 per cent, and phloroglucin 0.2 per cent.

Very large numbers of the organism were found on most of the substances that were ground and used as media. Wheat bran and ground Canada field pea seeds each had 10,000,000,000 organisms per gram at the end of three weeks. The organisms remained in a vigorous condition on these media for twenty weeks, as judged by the development of colonies and by the results of inoculation of the plants. In soy bean hay and soy bean roots no multiplication took place, and the organisms introduced at the time of seeding these two media were found to be dead at the end of three weeks.

First test of infecting power (*table 11*)

Three flowerpots were inoculated with each culture, these cultures being three weeks old when used. The inoculated plants were kept in the greenhouse and were examined for nodule development three weeks after inoculation. The data are presented in table 11:

TABLE 11. INFECTING POWER OF CULTURES THREE WEEKS OLD

Medium 334 with	Nodules in flower-pot 1	Nodules in flower-pot 2	Nodules in flower-pot 3
2.0 per cent cane sugar	Many	Many	Many
2.0 per cent dextrose	Many	Many	Few
2.0 per cent levulose	None	None	None
2.0 per cent lactose	Many	Many	Many
2.0 per cent galactose	Many	Many	Many
10.0 per cent cane sugar	Many	Many	Few
20.0 per cent cane sugar	Few	Few	Few
30.0 per cent cane sugar	Few	Few	Many
40.0 per cent cane sugar	Few	Few	Few
1.0 per cent salicin	Many	Many	None
0.5 per cent amygdalin	Many	Few	Few
0.5 per cent resorcin	None	None	Few
0.2 per cent phloroglucin	Few	Few	Few
0.5 per cent potassium oxalate	None	None	None
0.5 per cent potassium citrate	None	None	None
0.1 per cent potassium nitrate	Many	Many	Many
0.2 per cent potassium nitrate	Many	Many	Few
0.6 per cent potassium nitrate	Few	Few	Few
0.1 per cent calcium nitrate	Many	Many	Few
0.2 per cent calcium nitrate	Many	Many	Few
0.6 per cent calcium nitrate	Many	Many	Many
1.0 per cent Witte's peptone	Many	Many	Many
2.0 per cent Witte's peptone	Few	Few	None
5.0 per cent Witte's peptone	None	None	None
0.2 per cent Merck's peptone	Many	Many	Many
1.0 per cent Merck's peptone	Many	Many	Many
3.0 per cent Merck's peptone	None	None	None
Other media			
Soy bean hay	None	None	None
Soy bean roots	Few	Few	Few
Canada field pea hay	Few	Few	Few
Canada field pea roots	Many	Many	Many
Sandy soil	Many	Many	Many
Muck	Many	Many	Many
Sawdust	Many	Many	Many
Wheat bran	Many	Many	Many
Wheat middlings	Many	Many	Many
Corn meal	Many	Many	Many
Ground field peas	Many	Many	Many
Compost	Many	Many	Many
Partly decomposed cow feces	Many	Many	Many
Fresh cow feces	Many	Many	Many
Controls	None	None	None
Controls	None	None	None
Controls	None	None	None
Controls	None	None	None
Controls	None	None	None

As far as could be ascertained by these results, there was no reduction in the infecting power of the organisms when they were propagated and kept on the above media for three weeks. When these results are compared with the data in tables 9 and 10, it is seen that those cultures in which no visible growth took place produced either no inoculation or very poor inoculation, and that the cultures in which the organisms multiplied produced good inoculation.

Second test of infecting power (*table 12*)

In this test the cultures were ten weeks old. The exact number of nodules on each plant was counted, and from these figures calculations were made of (1) the total number of nodules in each flowerpot, (2) the average number of nodules per plant in each flowerpot, and (3) the average number of nodules per plant of all the plants inoculated with the same culture. It was hoped that the averages would give a more nearly accurate measure of the infecting power of the different cultures. The data are given in table 12. Fifteen plants were inoculated with each culture, five plants being grown in each flowerpot.

TABLE 12. INFECTING POWER OF CULTURES TEN WEEKS OLD

Laboratory number of culture	Medium in which *B. radicicola* was propagated	Laboratory number of flowerpots	Number of nodules on each plant					Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture
			Plant 1	Plant 2	Plant 3	Plant 4	Plant 5			
1	Medium 334 + 2 per cent cane sugar	1	14	4	1	0	12	31	6.2	8.6
		2	22	10	2	9	12	55	11.0	
		3	....	....	....	....	....	......	......	
2	Medium 334 + 2 per cent cane sugar	4	6	0	3	10	4	23	4.6	7.5
		5	12	4	15	12	10	53	10.6	
		6	14	7	6	2	7	36	7.2	
4	Medium 334 + 10 per cent cane sugar	7	5	7	14	12	12	50	10.0	10.2
		8	10	8	14	8	....	40	10.0	
		9	10	15	18	5	5	53	10.6	

TABLE 12 (*continued*)

Laboratory number of culture	Medium in which *B. radicicola* was propagated	Laboratory number of flowerpots	Number of nodules on each plant					Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture
			Plant 1	Plant 2	Plant 3	Plant 4	Plant 5			
6	Medium 334 + 20 per cent cane sugar	10	8	1	8	2	3	22	4.4	3.7
		11	15	5	1	0	5	26	5.2	
		12	4	0	4	0	0	8	1.6	
7	Medium 334 + 40 per cent cane sugar	13	11	8	9	5	....	33	8.2	9.2
		14	18	12	8	16	....	54	13.5	
		15	8	3	8	8	6	33	6.6	
8	Medium 334 + 2 per cent dextrose	16	0	14	15	10	9	48	9.6	8.7
		17	10	12	14	7	....	43	10.7	
		18	4	8	4	6	....	22	5.5	
9	Medium 334 + 2 per cent lactose	19	0	3	1	5	....	9	2.2	2.1
		20	3	1	0	2	3	9	1.8	
		21	0	6	5	0	0	11	2.2	
10	Medium 334 + 2 per cent galactose	22	5	8	7	....	....	20	6.7	9.7
		23	10	11	16	4	....	41	10.2	
		24	8	9	10	15	14	56	11.2	
12	Medium 334 + 2 per cent glycerin	28	3	2	16	14	14	49	9.8	12.1
		29	15	15	20	10	....	60	15.0	
		30	....	....	....	....	....	......	......	
13	Medium 334 + 0.5 per cent potassium oxalate	31	0	0	0	0	0	0	0	0.6
		32	0	2	3	4	....	9	2.2	
		33	0	0	0	0	0	0	0	
14	Medium 334 + 0.2 per cent phloroglucin	34	0	0	0	3	....	3	0.7	2.2
		35	0	0	0	0	....	0	0	
		36	1	3	2	5	15	26	5.2	
15	Medium 334 + 0.5 per cent resorcin	37	5	5	5	8	....	23	5.7	2.6
		38	1	4	0	6	....	11	2.7	
		39	0	0	0	0	0	0	0	
16	Medium 334 + 1 per cent salicin	40	0	2	4	0	3	9	1.8	3.0
		41	10	0	0	5	12	27	5.4	
		42	0	2	0	4	....	6	1.5	
17	Medium 334 + 0.5 per cent amygdalin	43	0	8	14	2	16	40	8.0	12.0
		44	10	15	24	15	8	72	14.4	
		45	14	20	10	....	....	44	14.7	

TABLE 12 (*continued*)

Laboratory number of culture	Medium in which *B. radicicola* was propagated	Laboratory number of flowerpots	Number of nodules on each plant					Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture
			Plant 1	Plant 2	Plant 3	Plant 4	Plant 5			
18	Medium 334 + 0.1 per cent asparagin	46	16	10	6	18	10	60	12.0	8.8
		47	10	5	8	10	....	33	8.2	
		48	6	0	12	2	10	30	6.0	
19	Medium 334 + 0.1 per cent potassium nitrate	49	7	3	10	12	15	47	9.4	6.9
		50	12	5	6	0	0	23	4.6	
		51	13	15	0	0	5	33	6.6	
20	Medium 334 + 0.2 per cent potassium nitrate	52	2	2	6	9	10	29	5.8	5.3
		53	1	8	8	12	1	30	6.0	
		54	0	2	1	8	10	21	4.2	
21	Medium 334 + 0.6 per cent potassium nitrate	55	10	16	10	18	2	56	11.2	10.6
		56	13	16	2	30	....	61	15.2	
		57	12	0	5	2	12	31	6.2	
22	Medium 334 + 0.1 per cent calcium nitrate	58	3	2	5	10	8	28	5.6	6.7
		59	6	6	3	10	8	33	6.6	
		60	8	0	14	5	12	39	7.8	
23	Medium 334 + 0.2 per cent calcium nitrate	61	12	12	10	5	14	53	10.6	13.1
		62	6	6	20	18	22	72	14.4	
		63	16	12	20	8	16	72	14.4	
24	Medium 334 + 0.6 per cent calcium nitrate	64	12	4	3	0	10	29	5.8	5.2
		65	3	2	7	7	9	28	5.6	
		66	0	0	6	10	5	21	4.2	
25	Medium 334 + 0.5 per cent potassium citrate	67	4	2	3	1	....	10	2.5	0.7
		68	0	0	0	0	0	0	0	
		69	0	0	0	0	0	0	0	
26	Medium 334 + 0.2 per cent Merck's peptone	70	4	7	8	4	4	27	5.4	4.4
		71	0	2	5	5	....	12	3.0	
		72	0	0	5	7	10	22	4.4	
27	Medium 334 + 1 per cent Merck's peptone	73	0	2	8	7	14	31	6.2	6.3
		74	20	7	0	0	8	35	7.0	
		75	7	8	0	12	2	29	5.8	
28	Medium 334 + 1 per cent Witte's peptone	76	14	0	10	0	15	39	7.8	15.8
		77	14	35	20	30	....	99	24.7	
		78	5	30	25	7	....	67	16.7	

TABLE 12 (*continued*)

Laboratory number of culture	Medium in which *B. radicicola* was propagated	Laboratory number of flowerpots	Number of nodules on each plant					Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture
			Plant 1	Plant 2	Plant 3	Plant 4	Plant 5			
29	Medium 334 + 2 per cent Witte's peptone	79	0	2	0	4	0	6	1.2	1.6
		80	2	3	4	0	1	10	2.0	
		81	0	1	5	0	....	6	1.5	
30	Medium 334 + 5 per cent Witte's peptone	82	0	0	0	0	0	0	0	0.4
		83	2	0	0	4	0	6	1.2	
		84	0	0	0	0	0	0	0	
31	Medium 334 + 5 per cent gelatin	85	10	14	16	35	34	109	21.8	13.9
		86	2	8	10	16	6	42	8.4	
		87	0	18	5	10	25	58	11.6	
32	Soy bean roots, ground	88	0	0	0	3	0	3	0.6	0.9
		89	0	0	1	7	....	8	2.0	
		90	2	0	0	0	0	2	0.4	
33	Soy bean hay, ground	91	0	0	0	0	0	0	0	0
		92	0	0	0	0	0	0	0	
		93	0	0	0	0	0	0	0	
34	Canada field pea roots, ground	94	0	0	5	8	6	19	3.8	7.0
		95	8	10	6	12	10	46	9.2	
		96	3	20	5	8	4	40	8.0	
35	Canada field pea hay, ground	97	12	5	2	8	....	27	6.7	2.6
		98	0	0	7	2	1	10	2.0	
		99	0	0	0	0	0	0	0	
36	Sawdust	100	1	0	0	0	0	1	0.2	1.6
		101	10	0	7	6	0	23	4.6	
		102	0	0	0	0	0	0	0	
37	Corn meal	103	0	0	0	0	0	0	0	0.1
		104	0	0	0	0	0	0	0	
		105	0	1	0	0	0	1	0.2	
38	Cornstarch	106	2	6	0	12	16	36	7.2	9.9
		107	18	6	2	12	....	38	9.5	
		108	7	15	8	15	20	65	13.0	
39	Wheat bran	109	18	0	8	8	6	40	8.0	8.6
		110	8	8	8	14	8	46	9.2	
		111	....	....	....	....	....	......	......	

TABLE 12 (*concluded*)

Laboratory number of culture	Medium in which *B. radicicola* was propagated	Laboratory number of flowerpots	Number of nodules on each plant					Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture
			Plant 1	Plant 2	Plant 3	Plant 4	Plant 5			
40	Wheat middlings	112	6	18	20	4	10	58	11.6	9.8
		113	20	12	5	6	10	53	10.6	
		114	12	0	7	3	14	36	7.2	
41	Compost	115	16	3	0	0	....	19	4.7	8.4
		116	12	4	15	8	0	39	7.8	
		117	10	14	12	16	8	60	12.0	
42	Partly decomposed cow feces	118	5	6	7	5	10	33	6.6	7.4
		119	6	3	14	8	12	43	8.6	
		120	8	6	3	4	14	35	7.0	
43	Fresh cow feces	121	0	0	7	0	....	7	1.7	8.4
		122	3	7	20	5	12	47	9.4	
		123	20	10	12	12	10	64	12.8	
44	Muck	124	10	8	10	12	10	50	10.0	7.1
		125	6	6	5	9	0	26	5.2	
		126	3	12	8	3	5	31	6.2	
45	Sandy soil	127	22	14	16	0	5	57	11.4	8.5
		128	10	0	8	4	6	28	5.6	
		129	....	....	....	....	....	......	......	
46	Controls	130	0	0	0	0	0	0	0	0.2
		131	0	0	0	0	0	0	0	
		132	0	3	0	0	0	3	0.6	
47	Controls	133	0	0	0	....	....	0	0	0
		134	0	0	0	0	....	0	0	
		135	0	0	0	0	0	0	0	

The average numbers of nodules per plant for all the plants inoculated with each of the given cultures appear in the last column in table 12. In table 13 these numbers are rearranged in numerical order according to the average number of nodules per plant produced by each culture:

TABLE 13. AVERAGE NUMBER OF NODULES PER PLANT IN SECOND TEST, ARRANGED IN NUMERICAL ORDER

Medium	Organisms living or not living*	Number of nodules per plant
Medium 334 + 1 per cent Witte's peptone	+	15.8
Medium 334 + 5 per cent gelatin	+	13.9
Medium 334 + 0.2 per cent calcium nitrate	+	13.1
Medium 334 + 2 per cent glycerin	+	12.1
Medium 334 + 0.5 per cent amygdalin	+	12.0
Medium 334 + 0.6 per cent potassium nitrate	+	10.6
Medium 334 + 10 per cent cane sugar	+	10.2
Cornstarch	+	9.9
Wheat middlings	+	9.8
Medium 334 + 2 per cent galactose	+	9.7
Medium 334 + 40 per cent cane sugar	+	9.2
Medium 334 + 0.1 per cent asparagin	+	8.8
Medium 334 + 2 per cent dextrose	+	8.7
Medium 334 + 2 per cent cane sugar	+	8.6
Wheat bran	+	8.6
Sandy soil	+	8.5
Compost	+	8.4
Fresh cow feces	+	8.4
Medium 334 + 2 per cent cane sugar	+	7.5
Partly decomposed cow feces	+	7.4
Muck	+	7.1
Canada field pea roots	+	7.0
Medium 334 + 0.1 per cent potassium nitrate	+	6.9
Medium 334 + 0.1 per cent calcium nitrate	+	6.7
Medium 334 + 1 per cent Merck's peptone	+	6.3
Medium 334 + 0.2 per cent potassium nitrate	+	5.3
Medium 334 + 0.6 per cent calcium nitrate	+	5.2
Medium 334 + 0.2 per cent Merck's peptone	+	4.4
Medium 334 + 20 per cent cane sugar	+	3.7
Medium 334 + 1 per cent salicin	0	3.0
Medium 334 + 0.5 per cent resorcin	0	2.6
Canada field pea hay	0	2.6
Medium 334 + 0.2 per cent phloroglucin	0	2.2
Medium 334 + 2 per cent lactose	+	2.1
Sawdust	+	1.6
Medium 334 + 2 per cent Witte's peptone	0	1.6
Soy bean roots	0	0.9
Medium 334 + 0.5 per cent potassium citrate	0	0.7
Medium 334 + 0.5 per cent potassium oxalate	0	0.6
Medium 334 + 5 per cent Witte's peptone	0	0.4
Controls (not inoculated)	0	0.2
Corn meal (culture contaminated)	0	0.1
Controls (not inoculated)	0	0
Soy bean hay	0	0

* + indicates living organisms; 0 indicates no living organisms.

In making any deductions from the preceding data, it must be remembered that the plants in this and in the other experiments were grown under special conditions. If the number of nodules on the plants in this experiment were dependent only on the degree of infecting power of the organism, an excellent illustration of variation in the infecting power would here be shown. This variation would, in this case, be due to the nature of the medium in which the organism was propagated.

That the number of nodules on a plant may be influenced by other factors than the infecting power of the organism has been shown in Part II of this paper, and also by experiments of other investigators. But the part that such other factors have played in this second test must be only a conjecture.

In order that a nodule may be produced, it is necessary that at least one organism shall come in contact with the root, that it shall enter the tissue of the root, and that it shall multiply inside the tissue. At least six factors can be mentioned which may have been of some importance in this experiment in bringing about this result:

1. *The distribution of the organisms through the entire volume of the soil in each flowerpot.*—The plant roots grow rapidly during the first three weeks after planting, and unless the organisms are evenly distributed through the soil a variation in the number of nodules might result if the plants are not allowed to grow for more than three weeks. Watering would tend to bring about an even distribution. All the flowerpots were watered twice each week, but the amount of water introduced into each pot was not measured — although it was uniformly constant — and because of this the distribution of the organisms throughout the soil may not have been uniform in all the flowerpots during the first two weeks.

2. *The number of organisms introduced into the different flowerpots at the time of inoculation.*—There is no available evidence to show how important this factor may be. That it may have exercised some influence seems highly probable. Reference to table 10 shows that there was a very great difference in the total number of organisms in the various cultures, and consequently the flowerpots inoculated with these cultures did not receive the same number of organisms.

3. *Multiplication of the organisms in the soil after inoculation.*—*Bacillus radicicola* multiplies readily in the sterilized soil that was used for growing the plants. This does influence the total number and the distribution

of the organisms in the soil. Moreover, if the infecting power of the organism were easily affected by the medium, it would be easily affected by the soil into which the organisms were introduced at the time of inoculation. The result would be that in course of time the infecting power of the different organisms, although different at the beginning, would become the same for all the organisms. If this is true, the difficulty in measuring the infecting power of a given culture is very evident.

4. *Resistance of the plant against the invasion of the organism.*—Nothing is known of this in connection with the leguminous plants and the nodule-forming organism. If such a character does exist in the plants, it probably varies in the different individuals and would influence the number of nodules formed.

5. *Infection from other sources than the inoculating material.*

6. *Infecting power of the organisms.*

It is evident from the above discussion that the number of nodules on plants may be the result of several factors operating simultaneously, and that it would be a difficult matter to determine their influence singly. The number of nodules, therefore, is not an accurate measure of the infecting power of the different cultures. Unfortunately, however, no other measure is available. In interpreting the above data, therefore, the limitations in the accuracy of the method must be borne in mind.

Comparing the data in table 13 with those in table 10, a close relation may be observed between the condition of the cultures when ten weeks old and the number of nodules on the plants inoculated with these cultures. The following cultures had no living organisms:

Medium 334 + 2 per cent Witte's peptone
Medium 334 + 5 per cent Witte's peptone
Medium 334 + 0.5 per cent resorcin
Medium 334 + 1 per cent salicin
Medium 334 + 0.5 per cent potassium oxalate
Medium 334 + 0.5 per cent potassium citrate
Medium 334 + 0.2 per cent phloroglucin (few organisms)
Soy bean hay
Soy bean roots
Canada field pea hay
Corn meal

Reference to table 13 shows that the plants inoculated with these cultures produced three nodules or less per plant. Whether the few nodules found resulted from contamination or from a few surviving organisms not detected by the plating can only be conjectured. The remaining cultures all produced good inoculation, with the exception of the sawdust and the lactose culture, the average number of nodules per plant varying between 3.7 and 15.8. To what extent this variation in the number of nodules is due to the infecting power of the different cultures it is difficult to say with certainty; but that a part of the variation is due to other causes is evident from the data in the next to the last column in table 12. In this column the figures represent the average number of nodules per plant for every flowerpot. Since three flowerpots were inoculated with each culture, the extent of variation in the number of nodules in these pots must be due to other causes than the infecting power. A number of the more pronounced cases of variation in the number of nodules in the three respective flowerpots which were inoculated by the same culture are shown in table 14:

TABLE 14. VARIATION IN AVERAGE NUMBER OF NODULES IN SOME OF THE FLOWERPOTS

Inoculated with culture number	Average number of nodules in		
	Flowerpot 1	Flowerpot 2	Flowerpot 3
28	24.7	7.8	16.7
31	21.8	8.4	11.6
21	15.2	6.2	11.2
17	14.7	8.0	14.4
7	13.5	6.6	8.2
38	13.0	7.2	9.5
43	12.8	1.7	9.4
18	12.0	6.0	8.2
41	12.0	4.7	7.8
45	11.4	5.6	....
1	11.0	6.2	....
8	10.7	5.5	9.6
2	10.6	4.6	7.2
44	10.0	5.2	6.2
34	9.2	3.8	8.0
6	5.2	1.6	4.4

The three flowerpots inoculated with culture 43 had 12.8, 1.7, and 9.4 nodules per plant, respectively. A similar relation exists between the other flowerpots considered in table 14. If the efficiency of these cultures were based on the number of nodules per plant, culture 43, for example, would be either very efficient or much reduced in infecting power, depending on which flowerpot was taken.

It seems apparent, from the preceding discussion, that it is extremely difficult to measure any variations in the infecting power of different cultures. When the great danger of infection of plants grown in sterile soil but not under sterile conditions is considered, and also the number of other factors that may affect nodule development on plants, not many clear-cut conclusions can be drawn from the second test. If the infecting power is measured by the number of nodules, by their size, by the uniformity of their distribution, and by their location, the following conclusions seem reasonable:

1. The cultures producing more than three nodules per plant are all efficient.

2. If one culture produced 3.7 nodules per plant and another culture produced 15.8 nodules per plant, the belief that the latter culture possesses greater infecting power than the former is not justified.

3. Some cultures produced no nodules, or only a few nodules confined to only one or two plants. Such cultures unquestionably lost their efficiency, but this loss of efficiency was parallel with the condition of the cultures. When no living organisms were found in a culture by using the plate method, such a culture produced no inoculation; and when living organisms were found, inoculation was produced.

4. Propagating and keeping *B. radicicola* of Canada field pea for ten weeks on media rich in nitrogenous matter, such as wheat bran, wheat middlings, fresh cow feces, and potassium and calcium nitrates, did not destroy the infecting power of the organism. If any injury to infecting power was caused by these substances, it could not be detected by the methods used in this experiment.

Third test of infecting power (*table 15*)

Cultures twenty weeks old were employed in this experiment. In inoculating the plants a known number of organisms was introduced into each flowerpot. By correlating the relation between the number of organisms used for the inoculation of each flowerpot and the number of nodules on the plants, it was hoped to find a more nearly exact measure of the infecting power of the organisms propagated in the different media. The results are summarized in table 15:

TABLE 15. INFECTING POWER OF CULTURES TWENTY WEEKS OLD

Laboratory number of culture	Medium in which *B. radicicola* was propagated	Laboratory number of flowerpots	Number of nodules on each plant							Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture	Number of organisms introduced into each flowerpot
			Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7				
1	Medium 334 + 2 per cent cane sugar....	1	10	6	5	2	8	....	....	31	6.2	4.5	324,000
		2	4	2	0	0	4	....	....	10	2.0		324,000
		3	8	14	0	0	....	....	....	22	5.5		324,000
3	Medium 334 + 2 per cent cane sugar	4	10	14	6	0	....	....	....	30	7.5	4.8	259,200
		5	6	1	0	0	....	....	....	7	1.7		259,200
		6	8	6	0	6	5	....	....	25	5.0		259,200
4	Medium 334 + 10 per cent cane sugar....	7	0	0	0	0	0	....	....	......	......	3.1	90,000
		8	0	0	0	0	0	5	....	5	8.3		90,000
		9	0	0	0	0	0	....	....	......	......		90,000
5	Medium 334 + 10 per cent cane sugar	10	0	5	4	6	....	....	....	15	3.7	1.4	5,000
		11	0	0	0	0	0	....	....	0	0		5,000
		12	0	0	3	0	3	0	....	6	1.0		5,000
6	Medium 334 + 20 per cent cane sugar	13	0	0	0	0	0	....	....	0	0	3.0	10
		14	0	0	0	6	7	....	....	13	2.6		10
		15	6	12	8	....	....	....	....	26	8.7		10
7	Medium 334 + 40 per cent cane sugar	16	0	0	0	0	0	0	0	0	0	0	0
		17	0	0	0	0	....	....	....	0	0		0
		18	0	0	0	....	....	....	....	0	0		0
8	Medium 334 + 2 per cent dextrose	19	8	4	4	0	....	....	....	16	4.0	4.4	52,000
		20	0	6	6	8	....	....	....	20	5.0		52,000
		21	4	....	....	....	....	....	....	4	4.0		52,000
9	Medium 334 + 2 per cent lactose	22	5	4	12	6	8	0	....	35	5.8	5.3	180
		23	8	6	0	0	4	0	3	21	3.0		180
		24	8	0	16	10	....	....	....	34	8.5		180
10	Medium 334 + 2 per cent galactose	25	12	10	6	16	....	....	....	44	11.0	5.9	60,000
		26	5	4	15	6	0	5	8	43	6.1		600
		27	0	0	0	3	4	7	....	14	2.3		600
11	Medium 334 + 2 per cent mannite	28	10	1	0	10	14	7	....	42	7.0	7.6	250,000
		29	12	5	2	8	2	....	....	29	5.8		2,500
		30	16	8	12	....	....	....	....	36	12.0		2,500
12	Medium 334 + 2 per cent glycerin	31	12	15	6	7	5	10	3	58	8.3	10.5	2,000,000
		32	8	14	15	15	16	10	8	86	12.3		20,000
		33	8	9	7	11	18	14	....	67	11.2		20,000
13	Medium 334 + 0.5 per cent potassium oxalate	34	0	0	0	0	0	....	....	0	0	0.3	0
		35	0	0	0	0	0	0	....	0	0		0
		36	0	0	0	5	....	....	....	5	1.2		0
14	Medium 334 + 0.2 per cent phloroglucin	37	0	0	0	0	0	0	....	0	0	1.6	0
		38	0	0	4	8	6	....	....	18	3.6		0
		39	....	....	....	....	....	....	....	......	......		0
15	Medium 334 + 0.5 per cent resorcin	40	0	0	0	0	....	....	....	0	0	0	0
		41	0	0	0	0	0	....	....	0	0		0
		42	0	0	0	0	0	....	....	0	0		0
16	Medium 334 + 1 per cent salicin	43	0	0	0	0	0	0	....	0	0	0	0
		44	0	....	....	....	....	....	....	0	0		0
		45	0	0	0	....	....	....	....	0	0		0

TABLE 15 (*continued*)

Laboratory number of culture	Medium in which *B. radicicola* was propagated	Laboratory number of flowerpots	Number of nodules on each plant							Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture	Number of organisms introduced into each flowerpot
			Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7				
17	Medium 334 + 0.5 per cent amygdalin	46	0	0	0	0	....	....	....	0	0	0	0
		47	0	0	0	0	0	....	....	0	0		0
		48	0	0	0	....	....	....	....	0	0		0
18	Medium 334 + 0.1 per cent asparagin	49	5	16	17	18	7	0	....	63	10.5	10.0	10,800,000
		50	6	15	12	10	6	....	....	49	9.8		108,000
		51	8	0	16	12	18	4	....	58	9.7		108,000
19	Medium 334 + 0.1 per cent potassium nitrate	52	25	17	6	0	....	....	....	48	12.0	9.5	7,200,000
		53	0	8	10	0	26	....	....	44	8.8		72,000
		54	20	12	10	4	4	0	....	50	8.3		72,000
20	Medium 334 + 0.2 per cent potassium nitrate	55	3	5	3	10	5	3	....	29	4.8	3.9	224,000
		56	5	5	0	0	....	....	....	10	2.5		224,000
		57	....	....	....	....	....	....	....	......	......		224,000
21	Medium 334 + 0.6 per cent potassium nitrate	58	0	10	12	4	14	0	....	40	6.7	6.7	14,400,000
		59	14	3	12	....	....	....	....	29	9.7		144,000
		60	6	10	0	2	....	....	....	18	4.5		144,000
22	Medium 334 + 0.1 per cent calcium nitrate	61	12	12	8	2	6	....	....	40	8.0	6.7	48,600,000
		62	2	1	7	9	5	3	....	27	4.5		486,000
		63	8	4	16	0	5	14	....	47	7.8		486,000
23	Medium 334 + 0.2 per cent calcium nitrate	64	2	1	8	14	12	16	....	53	8.8	8.6	10,800,000
		65	20	8	5	6	16	18	6	79	11.3		108,000
		66	5	3	6	14	3	8	1	40	5.7		108,000
24	Medium 334 + 0.6 per cent calcium nitrate	67	6	10	8	11	....	....	....	35	8.7	9.2	13,000,000
		68	3	7	8	9	11	7	8	53	7.6		130,000
		69	13	8	10	16	12	....	....	59	11.8		130,000
25	Medium 334 + 0.5 per cent potassium citrate	70	12	12	16	4	6	....	....	50	10.0	9.1	300,000
		71	18	8	4	20	....	....	....	50	12.5		3,000
		72	1	0	8	12	7	....	....	28	5.6		3,000
26	Medium 334 + 0.2 per cent Merck's peptone	73	1	0	1	12	10	0	....	24	4.0	5.9	800,000
		74	3	5	12	12	....	....	....	32	8.0		8,000
		75	8	0	0	14	6	10	....	38	6.3		8,000
27	Medium 334 + 1 per cent Merck's peptone	76	2	4	10	14	....	....	....	30	7.5	9.5	32,400,000
		77	10	12	15	18	4	....	....	59	11.8		324,000
		78	12	13	0	....	....	....	....	25	8.3		324,000
28	Medium 334 + 1 per cent Witte's peptone	79	3	7	3	0	5	....	....	18	3.6	9.4	21,600,000
		80	4	12	10	12	14	15	20	87	12.4		216,000
		81	12	8	16	....	....	....	....	36	12.0		216,000
29	Medium 334 + 2 per cent Witte's peptone	82	12	4	4	10	....	....	....	30	7.5	7.9	64,800,000
		83	4	0	12	8	....	....	....	24	6.0		648,000
		84	10	14	9	....	....	....	....	33	11.0		648,000
30	Medium 334 + 5 per cent Witte's peptone	85	0	0	0	2	0	0	....	2	0.3	0.1	0
		86	0	0	0	0	0	0	....	0	0		0
		87	0	0	0	0	0	0	0	0	0		0
31	Medium 334 + 5 per cent gelatin	88	3	2	2	10	7	6	....	30	5.0	6.6	6,480,000
		89	5	8	7	6	8	2	....	36	6.0		64,800
		90	7	8	12	14	5	....	....	46	9.2		64,800

TABLE 15 (*concluded*)

Laboratory number of culture	Medium in which *B. radicicola* was propagated	Laboratory number of flowerpots	Number of nodules on each plant							Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture	Number of organisms introduced into each flowerpot
			Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7				
32	Soy bean roots, ground	91	0	0	0	0	....	....	....	0	0	0	0
		92	0	0	0	0	....	....	....	0	0		0
		93	0	0	0	0	0	0	....	0	0		0
33	Soy bean hay, ground	94	0	0	0	0	0	....	....	0	0	0	0
		95	0	0	0	0	....	....	....	0	0		0
		96	0	0	0	0	0	0	....	0	0		0
34	Canada field pea roots, ground	97	0	0	0	0	0	0	0	0	0	0	0
		98	0	0	0	0	....	....	....	0	0		0
		99	0	0	0	0	....	....	....	0	0		0
35	Canada field pea hay, ground	100	0	0	0	....	....	....	....	0	0	0	0
		101	0	0	0	0	....	....	....	0	0		0
		102	0	0	0	0	0	0	....	0	0		0
36	Sawdust............	103	15	....	....	....	....	....	....	15	15.0	6.6	350,000
		104	4	9	5	5	3	....	....	26	5.2		3,500
		105	5	....	....	....	....	....	....	5	5.0		3,500
37	Corn meal...........	106	6	10	8	16	....	....	....	40	10.0	7.5	9,700,000
		107	1	2	3	12	5	14	....	37	6.2		97,000
		108	12	6	8	8	1	....	....	35	7.0		97,000
38	Cornstarch..........	109	12	7	14	....	....	....	....	33	11.0	9.1	9,700,000
		110	5	6	....	....	....	....	....	11	5.5		97,000
		111	8	7	14	....	....	....	....	29	9.7		97,000
39	Wheat bran.........	112	7	12	10	....	....	....	....	29	9.7	9.8	60,000,000
		113	1	2	22	8	....	....	....	33	8.2		600,000
		114	14	22	0	....	....	....	....	36	12.0		600,000
40	Wheat middlings.....	115	20	10	2	10	....	....	....	42	10.5	12.0	42,000,000
		116	15	12	16	....	....	....	....	43	14.3		420,000
		117	12	10	13	....	....	....	....	35	11.7		420,000
41	Compost............	118	12	14	8	0	....	....	....	34	8.5	7.3	12,000,000
		119	8	2	6	....	....	....	....	16	5.3		120,000
		120	9	4	10	....	....	....	....	23	7.7		120,000
42	Partly decomposed cow feces	121	5	4	5	....	....	....	....	14	4.7	8.3	324,000,000
		122	6	12	7	3	16	20	....	64	10.7		3,240,000
		123	6	0	18	12	2	....	....	38	7.6		3,240,000
43	Fresh cow feces......	124	10	12	7	15	....	....	....	44	11.0	9.4	81,000,000
		125	14	0	5	12	....	....	....	31	7.7		810,000
		126	....	....	....	....	....	....	....	......	......		810,000
44	Muck...............	127	4	0	0	0	....	....	....	4	1.0	3.5	24,000,000
		128	8	12	0	0	....	....	....	20	5.0		240,000
		129	1	8	7	2	....	....	....	18	4.5		240,000
45	Sandy soil...........	130	21	10	7	6	....	....	....	44	11.0	12.3	2,000,000
		131	17	14	16	2	....	....	....	49	12.2		20,000
		132	12	22	8	....	....	....	....	42	14.0		20,000

In this test a known number of organisms was introduced into each flowerpot at the time of inoculation, in order to trace the relation between the number of nodules developed and the number of organisms used for inoculation. It was hoped that in this way some means might be found of measuring not only the loss, but also the degree, of infecting power. From the data in table 15 it is seen that absolutely no relation exists between the number of organisms introduced into the flowerpots and the number of nodules that developed, so that the only measure of infecting power is the presence or the absence of nodules. This, however, does not measure the degree of infecting power, but only its presence or absence.

The average number of nodules per plant produced by each culture, as shown in table 15, are rearranged in numerical order in table 16. It is found on comparing tables 13 and 16 that the results of the third test of infecting power show a general agreement with the results of the second test.

If it is considered that the presence of three or more nodules per plant indicates that the culture was efficient, the following cultures are seen to have lost their efficiency:

Soy bean hay
Soy bean roots
Canada field pea hay
Canada field pea roots
Medium 334 + 10 per cent cane sugar
Medium 334 + 40 per cent cane sugar
Medium 334 + 0.2 per cent phloroglucin
Medium 334 + 1 per cent salicin
Medium 334 + 0.5 per cent amygdalin
Medium 334 + 0.5 per cent resorcin
Medium 334 + 0.5 per cent potassium oxalate
Medium 334 + 5 per cent Witte's peptone

The cultures that had any living organisms at the time of inoculation produced nodules practically in all cases in the three tests. The cultures in which no living organisms were found produced no nodules, or only a few unevenly distributed. It must be remembered that a certain amount of contamination may occur, and that the method of determining the presence of living organisms by plate cultures is not absolutely accurate. The cultures in this test were ten weeks older than those in the second test. The results show that the nodule-bacteria cultures can be kept for at least twenty weeks, and the bacteria will still be efficient in producing nodules.

TABLE 16. AVERAGE NUMBER OF NODULES PER PLANT IN THIRD TEST, ARRANGED IN NUMERICAL ORDER

Medium	Organisms living or not living*	Number of nodules per plant
Sandy soil	+	12.3
Wheat middlings	+	12.0
Medium 334 + 2 per cent glycerin	+	10.5
Medium 334 + 0.1 per cent asparagin	+	10.0
Wheat bran	+	9.8
Medium 334 + 1 per cent Merck's peptone	+	9.5
Medium 334 + 0.1 per cent potassium nitrate	+	9.5
Medium 334 + 1 per cent Witte's peptone	+	9.4
Fresh cow feces	+	9.4
Medium 334 + 0.6 per cent calcium nitrate	+	9.2
Cornstarch	+	9.1
Medium 334 + 0.5 per cent potassium citrate	+	9.1
Medium 334 + 0.2 per cent calcium nitrate	+	8.6
Partly decomposed cow feces	+	8.3
Medium 334 + 2 per cent Witte's peptone	+	7.9
Medium 334 + 2 per cent mannite	+	7.6
Corn meal	+	7.5
Compost	+	7.3
Medium 334 + 0.1 per cent calcium nitrate	+	6.7
Medium 334 + 0.6 per cent potassium nitrate	+	6.7
Medium 334 + 5 per cent gelatin	+	6.6
Sawdust	+	6.6
Medium 334 + 2 per cent galactose	+	5.9
Medium 334 + 0.2 per cent Merck's peptone	+	5.9
Medium 334 + 2 per cent lactose	+	5.3
Medium 334 + 2 per cent cane sugar	+	4.8
Medium 334 + 2 per cent cane sugar	+	4.5
Medium 334 + 2 per cent dextrose	+	4.4
Medium 334 + 0.2 per cent potassium nitrate	+	3.9
Muck	+	3.5
Medium 334 + 10 per cent cane sugar	+	3.1
Medium 334 + 20 per cent cane sugar	+	3.0
Medium 334 + 0.2 per cent phloroglucin	0	1.6
Medium 334 + 10 per cent cane sugar	+	1.4
Medium 334 + 0.5 per cent potassium oxalate	0	0.3
Medium 334 + 5 per cent Witte's peptone	0	0.1
Medium 334 + 40 per cent cane sugar	0	0
Medium 334 + 0.5 per cent resorcin	0	0
Medium 334 + 1 per cent salicin	0	0
Medium 334 + 0.5 per cent amygdalin	0	0
Canada field pea hay	0	0
Canada field pea roots	0	0
Soy bean hay	0	0
Soy bean roots	0	0

* + indicates living organisms; 0 indicates no living organisms.

EXPERIMENT 13

INFLUENCE OF MEDIA 300, 310, 335, AND 400

The organism used for this experiment was propagated on medium 335, and had been kept in the laboratory for two years and three months, where it was exposed to diffused light and transfers had been made at intervals. When the experiment was started the organism readily produced nodules on the plants, showing that its infecting power had not been lost.

The plan of the experiment was to propagate the organism on both the nitrogen-free and the nitrogenous media for a period of time, and then to test these cultures for nodule production. The following procedure was adopted: Media 300, 310, 335, and 400 were introduced into test tubes, sterilized, and sloped. Four slopes from each of these media were inoculated with the same culture of the organism. A few days later a second set of four slopes from each medium was inoculated, and so on. Nine such sets of agar slopes from each of the four media were inoculated in one hundred and fifteen days. The four agar slopes of the same medium in each set were inoculated from one of the four slopes of the same medium from the previous set, so that on all the slopes of the same medium in the nine sets the organism was under the influence of the same media for one hundred and fifteen days. The age of the agar slopes, however, differed in the respective sets. When the ninth set was inoculated, four additional tubes of media 300, 310, and 400 were inoculated with a culture of the organism which up to that time had been propagated on nitrogen-free medium 335. These test-tube cultures are designated in table 17 as set X. They were fifteen days old and had been under the influence of nitrogenous media for only fifteen days when tested for infecting power. In order to prevent the effects of drying, melted paraffin was poured on the cotton plugs after a good growth had developed. All the cultures were kept at room temperature.

The infecting power of these agar-slope cultures was tested by inoculating Canada field peas. The method of growing and examining the plants was the same as in experiment 12. In inoculating the plants, the growth from each agar slope was introduced into 100 cubic centimeters of sterile water, the number of organisms in this was determined by the plate method, and a definite quantity of this infusion was poured over the seeds in each flowerpot.

Unfortunately, an accident happened to a large number of the flowerpots in the greenhouse, and consequently the data are not complete. As far as they could be obtained, they are summarized in table 17:

TABLE 17. INFLUENCE OF MEDIA 300, 310, 335, AND 400

Laboratory number of culture	Number of set	Medium	Age (in days) of culture when used for inoculation	Laboratory number of flowerpots	Number of nodules on each plant						Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture	Number of living organisms found in the culture
					Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6				
1	I	335	115	1	0	6	14	7	6	6	39	6.5	9.8	300,000,000
				2	13	15	12	7	8	....	55	11.0		
				3	25	16	2	....	....	....	43	14.3		
2	I	300	115	4	0	0	0	0	0	0	0	0	0	0
				5	0	0	0	0	....	....	0	0		
				6	0	0	0	....	....	....	0	0		
3	I	400	115	7	0	0	0	0	0	0	0	0	0	0
				8	0	0	0	0	0	0	0	0		
				9	0	0	0	0	0	0	0	0		
4	II	335	110	10	12	13	8	8	5	....	46	9.2	7.3	350,000,000
				11	6	8	8	5	5	2	34	5.7		
				12	....	....	....	....	....	....	......	......		
5	II	300	110	13	0	0	0	0	0	0	0	0	0	0
				14	0	0	0	0	0	0	0	0		
				15	0	0	0	0	....	....	0	0		
6	II	400	110	16	0	0	0	0	0	....	0	0	0.6	0
				17	0	0	0	0	0	4	4	0.7		
				18	0	0	0	0	6	....	6	1.2		
7	III	335	105	19	8	9	12	20	14	....	63	12.6	9.5	360,000,000
				20	9	20	6	7	4	....	46	9.2		
				21	8	10	7	8	0	....	33	6.6		
8	III	300	105	22	0	0	0	0	0	0	0	0	0	0
				23	0	0	0	0	0	0	0	0		
				24	0	0	0	0	0	0	0	0		
9	III	400	105	25	0	0	0	0	0	0	0	0	0	0
				26	0	0	0	0	0	0	0	0		
				27	0	0	0	0	0	0	0	0		
10	III	310	105	28	12	5	15	4	10	15	61	10.2	10.7	144,000,000
				29	14	16	6	18	8	13	75	12.5		
				30	7	10	6	12	....	....	35	8.7		
11	IV	400	98	31	0	2	0	0	....	....	2	0.5	0.5	90,000
				32	....	....	....	....	....	....	......	......		
				33	....	....	....	....	....	....	......	......		
12	VI	335	81	34	13	1	4	17	6	....	41	8.2	6.9	180,000,000
				35	4	2	14	8	2	5	35	5.8		
				36	14	10	6	0	8	3	41	6.8		
13	VI	300	81	37	0	0	0	0	0	0	0	0	0	0
				38	....	....	....	....	....	....	......	......		
				39	....	....	....	....	....	....	......	......		
14	VI	400	81	40	10	11	12	7	0	....	40	8.0	8.5	1,000,000
				41	0	20	6	7	13	8	54	9.0		
				42	12	10	6	6	....	....	34	8.5		
15	VI	310	81	43	20	15	8	0	....	....	43	10.7	10.7	540,000,000
				44	....	....	....	....	....	....	......	......		
				45	....	....	....	....	....	....	......	......		

TABLE 17 (*concluded*)

Laboratory number of culture	Number of set	Medium	Age (in days) of culture when used for inoculation	Laboratory number of flower-pots	Number of nodules on each plant						Total number of nodules in each flowerpot	Average number of nodules per plant in each flowerpot	Average number of nodules per plant produced by the culture	Number of living organisms found in the culture
					Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6				
16	VII	335	61	46	12	0	6	8	0	16	42	7.0	9.8	396,000,000
				47	16	7	15	6	20	....	64	12.8		
				48	3	20	0	6	22	9	60	10.0		
17	VII	400	61	49	2	10	4	....	....	....	16	5.3	7.7	10,000,000
				50	5	5	12	16	....	....	38	9.5		
				51	....	....	....	....	....	....	......	......		
18	VII	310	61	52	0	0	0	0	0	0	0	0	0	0
				53	0	0	0	0	0	0	0	0		
				54	0	0	0	0	0	0	0	0		
19	VIII	335	49	55	0	10	8	9	0	....	27	5.4	6.2	216,000,000
				56	8	5	0	....	....	....	13	4.3		
				57	8	10	9	7	....	....	34	8.5		
20	VIII	300	49	58	7	9	....	....	....	....	16	8.0	11.2	16,000,000
				59	6	7	18	7	16	12	66	11.0		
				60	6	13	30	4	....	....	53	13.2		
21	VIII	400	49	61	8	0	2	18	0	....	28	5.6	6.8	252,000,000
				62	12	13	4	4	14	....	47	9.4		
				63	4	0	1	6	16	....	27	5.4		
22	VIII	310	49	64	0	6	25	....	....	....	31	10.3	7.8	8,000,000
				65	6	10	6	5	....	....	27	6.7		
				66	12	2	0	10	12	....	36	7.2		
23	IX	335	15	67	10	6	5	2	8	....	31	6.2	4.5	576,000,000
				68	4	2	0	0	4	....	10	2.0		
				69	8	14	0	0	....	....	22	5.5		
24	IX	300	15	70	12	35	23	0	....	....	70	15.0	8.5	4,320,000,000
				71	18	0	0	13	5	8	44	7.3		
				72	0	7	0	2	5	....	14	2.8		
25	IX	400	15	73	0	5	8	0	....	....	13	3.2	5.8	2,592,000,000
				74	10	4	4	2	7	10	37	6.2		
				75	7	18	0	....	....	....	25	8.3		
26	IX	310	15	76	9	5	4	7	0	0	25	4.2	6.2	3,240,000,000
				77	8	0	4	7	....	....	19	4.7		
				78	12	8	8	15	0	12	55	9.2		
27	X	300	15	79	5	0	0	12	6	....	23	4.6	7.1	180,000,000
				80	4	7	30	20	12	....	73	14.6		
				81	0	0	3	3	5	....	11	2.2		
28	X	400	15	82	2	15	2	8	6	....	33	6.6	7.1	972,000,000
				83	5	9	1	6	6	8	35	5.8		
				84	6	8	0	25	6	8	53	8.8		
29	X	310	15	85	6	0	0	7	7	4	24	4.0	5.2	1,152,000,000
				86	0	0	0	0	7	10	17	2.8		
				87	12	25	0	....	....	....	37	12.3		

Results

The nature and the composition of the four media used in experiment 13 are given in table 1, page 11. No nitrogen in any form was added to medium 335. The other three media received 0.3 per cent of Liebig's beef extract, and, in addition to this, media 300 and 310 received 1 per cent and medium 400 received 2 per cent of Witte's peptone. Medium 335 contained 2 per cent of cane sugar, and medium 310 contained 2 per cent of dextrose.

As seen from table 17, the age of the individual cultures varied between fifteen days and one hundred and fifteen days; but the organisms in all the cultures, except 27, 28, and 29, had been under the influence of their respective media for one hundred and fifteen days. Cultures 27, 28, and 29 were fifteen days old and had been under the influence of their respective media for only fifteen days, having been grown previously on medium 335.

The influence of these four media on the organism is shown in table 17. In order to bring out the relations more clearly, a part of these data are rearranged in table 18:

TABLE 18. INFLUENCE OF AGE OF CULTURES ON THE GIVEN MEDIA

Laboratory number of culture	Culture medium	Age of culture (days)	Average number of nodules per plant	Number of living organisms in culture
23	335	15	4.5	576,000,000
19	335	49	6.2	216,000,000
16	335	61	9.8	396,000,000
12	335	81	6.9	180,000,000
7	335	105	9.5	360,000,000
4	335	110	7.3	350,000,000
1	335	115	9.8	300,000,000
24	300	15	8.5	4,320,000,000
20	300	49	11.2	16,000,000
13	300	81	0	0
8	300	105	0	0
5	300	110	0	0
2	300	115	0	0
25	400	15	5.8	2,592,000,000
21	400	49	6.8	252,000,000
17	400	61	7.7	10,000,000
14	400	81	8.5	1,000,000

TABLE 18 *(concluded)*

Laboratory number of culture	Culture medium	Age of culture (days)	Average number of nodules per plant	Number of living organisms in culture
11	400	98	0.5	90,000
9	400	105	0	0
6	400	110	0.6	0
3	400	115	0	0
26	310	15	6.2	3,240,000,000
22	310	49	7.8	8,000,000
18	310	61	0	0
15	310	81	10.7	540,000,000
10	310	105	10.7	144,000,000
27	300	15	7.1	180,000,000
28	400	15	7.1	972,000,000
29	310	15	5.2	1,152,000,000

The organism had been propagated and kept on medium 335, a non-nitrogenous medium, for over two years before this experiment was started. Several tests of infecting power were made during that time. This medium was used also in experiment 12. As far as could be judged by all these tests, the infecting power of the organism remained constant when propagated and kept on this medium. Therefore the results shown in experiment 13 as to the infecting power of the cultures on medium 335 are taken to represent the normal infecting power of the organism, and serve as a basis for comparison of the infecting power of the cultures on the other media.

Of the seven cultures on medium 300, a nitrogenous medium, three cultures — namely, 20, 24, and 27 — produced nodules. Cultures 24 and 27 were fifteen days old and culture 20 was forty-nine days old. The organisms in cultures 20 and 24 were under the influence of medium 300 for one hundred and fifteen days, and culture 27 for only fifteen days. These three cultures appeared as effective as those on medium 335. The remaining cultures on medium 300 were from eighty-one to one hundred and fifteen days old and did not produce any nodules, apparently having lost their power of infection.

Similar results were obtained with the nitrogenous medium 400. Of the nine cultures on this medium, five produced nodules, two were doubtful, and two produced no nodules. Culture 28 was fifteen days old and had been under the influence of this medium for only fifteen days; while culture 25 was fifteen days old and had been under the influence of this medium for one hundred and fifteen days. No appreciable difference in the infecting power of these two cultures was observed.

Medium 310 is the same as medium 300 except that it contains 2 per cent of dextrose. Five of the six cultures on this medium produced nodules; one did not, and that was not the oldest culture. Comparing these results with those from medium 300, it seems as if the dextrose protected the infecting power of the organism on this medium.

In the last column of Table 18 the number of living organisms in each culture is given, and in the next to the last column the number of nodules per plant produced by each culture is given. It is seen that in all cases in which no living organisms were found in a culture no nodules were produced by that culture, and that the cultures in which living organisms were present produced nodules. Culture 6 is the only exception, but in this case only two plants out of sixteen had four and six nodules, respectively, and the size and location of these nodules pointed to later contamination.

According to the above data, *Bacillus radicicola* of Canada field pea does not lose its infecting power when propagated on medium 300 and 400 for one hundred and fifteen days. Some of the cultures on these two media did lose their infecting power, but in all cases these were the older cultures. This loss in efficiency is due to death of the culture, and death is induced by nitrogenous media after a considerable duration.

SUMMARY

1. The causal organism in the case of Canada field pea nodules is *Bacillus radicicola.* Its flagella are peritrichic, and eight was the largest number found. Its group number is B. 222.2322033.

2. Nodules developed both in light and in darkness. A larger number of nodules, however, developed in darkness.

3. Nodules developed readily both in the soil extract and in synthetic nutrient solutions in which the nitrates were either omitted or replaced by chlorides. The nodules continued to increase in number as long as the plants continued to grow.

4. In a full nutrient solution containing nitrates a few nodules may develop immediately after inoculation, but a subsequent continual development of nodules seems to be inhibited.

5. No nodule development took place in nutrient solutions in which the individual essential elements were omitted, except in the case of nitrogen.

6. In sandy soil a moisture content of 20 to 40 per cent was more favorable for nodule development than lower or higher percentages.

7. The addition of KNO_3, $Ca(NO_3)_2$, NH_4Cl, $FeCl_3$ KCl, or peptone to sandy soil in the proportion of $\frac{1}{4}$ gram of the salts to 300 grams of the soil, air-dry, had an inhibiting effect on nodule development on Canada field peas. The addition of $MgSO_4$, KH_2PO_4, $Ca(H_2PO_4)_2$, and tannic acid, especially at the lower concentrations, in 300 grams of the soil, had a beneficial effect on nodule development on Canada field peas.

8. Nutrition markedly influences the morphology of the nodule organisms.

9. The addition of 1 cubic centimeter or more of a normal solution of HCl to 10 cubic centimeters of agar medium 334, 335, or 337 was injurious to the vitality, and therefore to the infecting power, of the alfalfa-nodule organism. The addition of 2 cubic centimeters or less of a normal solution of NaOH to 10 cubic centimeters of each of the above media seemed to be slightly beneficial to the vitality and the infecting power of this organism.

10. The organism of Canada field peas produced no visible growth in medium 334 when the following substances were added: levulose 2 per cent, phloroglucin 0.2 per cent, potassium oxalate 0.5 per cent, potassium citrate 0.5 per cent, Witte's peptone 5 per cent, Merck's peptone 3 per cent.

11. The organism multiplies readily in some soils and in various substances; as many as 10,000,000,000 organisms per gram developed in wheat bran and in ground peas.

12. The infecting power of *B. radicicola* of Canada field pea was not affected after the organism had been kept on medium 335 for two years and a half in the laboratory, the culture being transferred once each month.

13. The infecting power of the organism was not appreciably influenced by the various media. All the cultures in which living nodule-forming organisms were found at the time of trial produced nodules.

14. In some media and under certain conditions the organisms died much sooner than in other media. The nitrogenous media did not seem to influence the infecting power of the organism.

15. It is not difficult to determine whether or not a given culture can produce nodules, but there is no accurate method of measuring the slight variations in infecting power that may exist in the different cultures.

GENERAL DISCUSSION

The definition of *virulence* as given by various authors in the medical texts is not clear, but in general the word is defined as meaning the power of microorganisms to invade and multiply in the tissues of a host and cause some injury or disease to the host. It was found by Peirce (1902), Fred (1911), and others, that the cells in the nodule are injured by the nodule-forming organism and become abnormal, and that for this reason the relation between the microorganism and the legume is a parasitic one. Furthermore, a nodule on a leguminous plant is a swelling, a hypertrophic formation; and morphologically speaking, it is an abnormality, or a form of disease. Nodule formation, therefore, may be considered as of a pathological nature. But, it may be asked, which is the normal root of the legume — the one with the nodules, or the one without the nodules, or both? Whatever the morphological and cytological evidence may be, one well-established fact stands out; namely, that leguminous plants are benefited by the presence of nodules. No positive evidence has been produced thus far to show that the microorganism which causes nodules is injurious to leguminous plants. The nodule-forming organism penetrates the root tissue, multiplies therein, and apparently derives its necessary food therefrom; and in return for this it enables the plant to obtain a certain amount of nitrogen. There is, therefore, a mutual and beneficial exchange and the relation is symbiotic.

The primary object of the experiments reported in this investigation was to determine whether the power of the nodule-forming organism to cause nodules is easily altered by artificial media. The nodule-forming organism of Canada field pea was chosen for this purpose, and the results apply only to that organism. It is probable that the organisms from other legumes might have given different results. Since the value of the investigation depended so much on the securing of a pure culture, the various precautions, as previously indicated, were adopted for this purpose.

The fact that a pure culture is obtained from a nodule and resembles in its cultural characteristics the true nodule-forming organism is not a sufficient proof that such an organism is the nodule-forming organism. De' Rossi (1907), in his investigation, emphasizes this point, and unquestionably the information on the general subject of nitrogen fixation by leguminous plants has been colored by results from experiments in which some organism other than the nodule-forming organism was employed.

As stated on page 59, the number, size, and location of the nodules on the roots are probably influenced by a number of factors. Since the number of nodules produced on the plants in a given time was used as the measure of the infecting power of the culture with which the plants were inoculated, it seemed advisable to study the influence of several factors and thus to determine whether Canada field peas readily form nodules under the conditions that it was planned to use in Part III. The results of these experiments in Part II tend to point to the conclusion that, in general, the conditions favoring the normal development of plants favor also the development of nodules. An exception to this is found in the fact that the presence of nitrates tends to inhibit the development of nodules, and at the same time favor the normal development of the plants. No satisfactory explanation for this phenomenon has as yet been given. The plants appear not to be injured by the presence of nitrates, and neither do the nodule-forming organisms seem to be injured when propagated on a medium in which nitrate is present (see experiment 13). The explanation that the plants are made more vigorous when supplied with nitrogen, and can more readily resist the invasion of the microorganisms, cannot be taken seriously. It might be noted here that the root system of Canada field peas grown in Pfeffer's nutrient solution appears normal, tending to become slightly brownish; but when the nitrate in the same solution is replaced by the chloride of the same metal, the root system becomes larger, the roots being more numerous and longer. Consequently, the rate of growth of the root tissue is accelerated by the absence of nitrates. Whether this somewhat rapid growth of the root tissue has any relation to nodule formation is not known. It is highly probable that a biological factor also influences the development of nodules. The microbial flora of the soil or of the solution in which the plants are grown is undoubtedly influenced by the composition of that soil or solution. The microorganisms that thrive best in a highly nitrogenous soil or solution, and the products of their metabolic activities, may exercise an injurious influence on the nodule organisms and thus prevent them from multiplication and distribution through the soil.

The influence of artificial media on the power of *Bacillus radicicola* to cause nodules is of considerable importance, in view of the fact that inoculation for leguminous crops with pure cultures is extensively practiced and the pure cultures have to be propagated on some media. Frank (1899), in commenting on the low efficiency of nitragin, suggested that probably the medium (gelatin) on which the cultures were propagated and kept was not favorable for the organisms, and that this might be the cause of the low efficiency of nitragin. Hiltner (1900) finally substituted liquid media for the gelatin, and was able to obtain better results from the legume inoculation. Süchting (1904) found that not only the gelatin media are injurious to the bacteria — a point shown by Hiltner — but the agar media also may be unfavorable. Moore (1905) made the observation that the nodule-forming bacteria increase most rapidly on a medium rich in nitrogen, but that the resulting growth is usually very much reduced in infecting power. Lewis and Nicholson (1905), on the other hand, state that the presence or absence of nitrogen in the culture media is not the determining factor in maintaining the activity of the germ.

A reasonable conclusion from the investigations mentioned above seems to be that some artificial media are more favorable than others for the propagation of *B. radicicola*, and that the amount of growth is not always directly proportional to the nodule-producing efficiency of the organism. With this conclusion the experiments in Part III are in accord. In addition to this, the experiments point to the conclusion that *B. radicicola* of Canada field pea does not possess "virulence" in a pathological meaning of the word. The ability to cause nodules is so closely bound up with the general vitality of the bacteria that our means and methods cannot detect any variations, if such there are, in their nodule-producing ability. The writer's opinion is that every living nodule-producing organism in a vigorous condition, will, if given a chance, cause nodule development no matter on what kind of media it has been propagated. The propagation of the organisms on different media does not measurably affect their nodule-producing efficiency. The organisms die sooner on some media than on others, and the loss of the nodule-producing efficiency of the cultures is due to the dying-out of the organisms. Whether the injurious agent is some ingredient of the media, or lack of a proper nutrient, or the accumulation of the metabolic products, has not been determined. The observation that a copious growth is usually of reduced nodule-producing efficiency suggests the last as the probable, or at least a partial, explanation.

When one examines leguminous plants grown under favorable conditions, one finds that each plant has a limited number of nodules and that the number and the size of the nodules vary on the different plants. Hiltner (1900), holding the relation between *B. radicicola* and the leguminous plants to be of a pathological nature, concluded from his experiments that the variableness of the infecting power of the nodule-forming bacteria is the limiting factor which determines the number and size of the nodules under otherwise favorable conditions. Süchting (1904), in trying to account for the limited number of nodules on each plant, emphasized the resistance of the plants against the invasion of the bacteria. His explanation is as follows: "In contrast to Hiltner's immunity theory, I am of the opinion that the nodule formation and their number are regulated by the relation between the antibodies in the plant and the infecting substance of the bacteria." [7]

These explanations do not appear to the writer to explain the conditions. No pathological explanation can account for a limited number of nodules. The physical condition and the chemical composition of the soil, the amount of moisture, and the microbial flora of the soil, are some of the important factors that interfere, directly or indirectly, with the coming together of *B. radicicola* and the roots of the plants. The stage in the development of the plant, the rate of growth of the roots, the number of root hairs, the character of the root tissue, and other factors, may also play a part in limiting the development of nodules.

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[7] Translation from the original German.

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FUSARIA OF POTATOES

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FUSARIA OF POTATOES

FUSARIA OF POTATOES[1]

C. D. Sherbakoff

INTRODUCTORY

The purpose of this work has been to lay down a basis for the study of the disease known as the fusarial wilt and dry rot of potatoes, *Solanum tuberosum*.

On the basis of previous work done by various mycologists and plant pathologists on Fusaria of potatoes, it was evident, even before the monographic work of Appel and Wollenweber (1910), that the potato is a host of a number of different species of Fusarium. At the same time the descriptions of these organisms were, almost without exception, so confusing, and the organisms as a rule were apparently so variable, that there was no sure way of identifying a Fusarium isolated from potato with a previously described Fusarium even of the same host. Such a state of affairs led Smith and Swingle (1904:50) to make a general statement as follows: "Many specific names have been given to *Fusaria* growing on the potato, and while some of these names may stand for distinct forms, this is not at all certain. Most of these names undoubtedly are synonyms, and for the purpose of this paper all are regarded as such."

On the other hand, Lindau (1908) and others continued to believe firmly that there were different Fusaria of potatoes, and that *Fusarium oxysporum* as described by Smith and Swingle is nothing else than a "Mischart."

Whether more than one species of Fusarium occurs on this host and, if more than one, on what basis they could be separated, was then the question which in the opinion of the writer needed to be definitely settled before any pathological work with these organisms could be properly done. This involved the accumulation of as many different strains of the Fusaria as was practicable, from all available sources, and their comparison in pure culture on various suitable media and under the same conditions of growth. On this basis the work has been carried on since the autumn of 1911.

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[1] Presented to the Faculty of the Graduate School of Cornell University as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

When the work was well under way and it became apparent that there are indeed a considerable number of species of Fusarium, it came to the writer's knowledge that the same thing was definitely proved, at least for European conditions, by Appel and Wollenweber (1910) in their *Grundlagen* (page 105), in which they state that most of the Fusaria described by them were isolated from the potato, mainly from the tubers. Since then this work has been continued with still greater confidence in its underlying principles, but now mainly in order to verify the European findings for the American conditions.[2]

All cultures of the Fusaria were obtained by isolations made from diseased parts of the potato plant, mainly from tubers received from pathologists in nearly every experiment station in the United States. Of all the strains of the Fusarium-like organisms thus obtained, over one hundred and twenty organisms at first seemed to differ in some way or another. After a careful comparative study of all these strains, more than half of the number proved to be identical with others.

Some of the remaining organisms were identified with several Fusaria described by Appel and Wollenweber (1910), by Jamieson and Wollenweber (1912), and by Wollenweber (1913, b and c). These are as follows:

Fusarium affine Faut. et Lamb.
F. coeruleum (Lib.) Sacc.
F. dimerum Penz.
F. discolor Ap. et Wr.
F. Martii Ap. et Wr.
F. metacroum Ap. et Wr.
F. oxysporum Schlecht., as described by Wollenweber
F. radicicola Wr.
F. Solani (Mart. p. par.) Ap. et Wr.
F. subulatum Ap. et Wr.
F. trichothecioides Wr.

Still other organisms were found to be very closely related either to some of the species named above or to certain other species, at the same time differing sufficiently from them to be separated on a conservative basis from those species. These organisms were then designated as new varieties of the following old species:

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[2] The general discussion of the genus Fusarium was known to the writer before he began his work, when in the summer of 1911 Dr. Jensen received the *Grundlagen* and now and then read parts of it to the writer. Having no idea that Appel and Wollenweber were working mainly with the Fusaria of the potato, the writer did not study their work thoroughly as early as he should have.

Fusarium caudatum Wr. (one new variety)
F. culmorum (W. Smith) Sacc. (one new variety)
F. discolor Ap. et Wr. (one new variety)
F. falcatum Ap. et Wr. (one new variety)
F. Martii Ap. et Wr. (two new varieties)
F. metacroum Ap. et Wr. (one new variety)
F. oxysporum Schlecht. (three new varieties)
F. redolens Wr. (one new variety)
F. Solani (Mart. p. par.) Ap. et Wr. (two new varieties)
F. subulatum Ap. et Wr. (one new variety)
F. udum (Berk.) Wr. (one new variety)

The remaining organisms of the genus Fusarium were then named as new species and their varieties. It is probable that at least some of them have been previously described, but the descriptions given are so incomplete that any reasonable identification is doubtful. The matter of identification is discussed on another page.

Besides the Fusaria, several organisms were isolated in the course of the work which more or less closely resemble Fusarium though belonging to a different genus (Ramularia) of the Hyphomycetes. As these organisms are often found on the potato and are easily confused with species of Fusarium, they are treated here, but of course under their proper generic name.

On the whole the work, with a few exceptions, fully verifies the principles laid down by Appel and Wollenweber (1910). The chief exceptions are as follows: (1) The Fusaria as such can be distinguished on almost any medium, including artificial media, provided that the medium is not extremely poor or rich in food materials and also provided that the moisture supply in the medium is well regulated. (2) The microconidia should be recognized as a special kind of spores because of their importance in classification of these fungi. Though microconidia genetically do not represent a separate kind of spores, nevertheless their typical absence for certain closely related Fusaria, as well as their typical presence for others, is characteristic. Besides, whenever they are present they have a constant and often distinctive type of their own. (3) No typical coremial nor typical pionnotal form of fructification was observed in any of the true Fusaria, though types of fructification resembling those forms more or less closely were common.

SCOPE OF WORK

The work was confined to a study of the Fusaria as such, that is, without any consideration of the possible perfect forms.[3] The study was centered mainly on the characters that seem to be most important and practicable for a natural classification of these organisms. Thus, especial attention was paid to the presence and type of each kind of spores (micro- and macroconidia and chlamydospores). Type of color production was found to be next in importance. Type of fructification and of conidiophores, size of spores, presence or absence and kind of sclerotia, and rate and character of colony growth, were also found to be of more or less considerable importance for differentiation of the species.

Some work has been done on the action of these organisms under different environmental conditions and also with respect to their pathogenicity. In regard to the latter the following four facts should be mentioned:

1. Extensive inoculations of potato plants with all the Fusaria presented here yielded negative results and would indicate that they are not wilt producers.[4]

2. Several series of inoculations of potato tubers showed (a) that a considerable number of the Fusaria can cause more or less rapid decay of the tubers, and (b) that most of the Fusaria readily produce rot only after the tubers begin to sprout.

3. The commonest rot-producing organism, at least in eastern United States, is *F. coeruleum* (Lib.) Sacc.

4. The inoculations into tubers seem to indicate that some species widely different morphologically may act similarly pathologically, and that other species very closely related from the morphological standpoint differ very widely in their pathogenicity.

The other results of this phase of the work will be mentioned only in those instances in which different action under different environment is of importance in identification.

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[3] In this study no special attempt was made to discover the perfect stage of any of these organisms. At the same time it is to be noted that under the condition of the work when a considerable number of "natural" as well as "artificial" media were used, none of the Fusaria produced a perfect stage.

[4] The writer does not mean by this statement that the Fusaria actually never produce wilt of potatoes, because it is possible that the negative results were due to lost virulence of the cultures used or to some other important factor that escaped attention.

SOURCE AND METHODS OF ISOLATION

Most of the species of Fusarium and related organisms presented here were isolated from potato tubers affected with dry or soft rot. A number of isolations were made also from discolored fibro-vascular bundles of potato tubers and from stems of wilted potato plants. A single organism, *Fusarium metacroum* var. *minus*, was isolated from a spot on the surface of a half-dead potato stem.

In a few cases the isolations were made by means of poured plate dilutions, but in the majority of cases they were made from affected tissues of the host. The affected part of the host was first thoroughly wiped with a piece of cheesecloth moistened in 0.1 per cent solution of corrosive sublimate, and then the "skin" was peeled off just above the affected part, or the diseased part of the plant was broken open so that the spot from which the isolation was to be made was not touched even with sterile utensils.[5] Four or five small fragments of diseased tissue were cut out with a sterile scalpel and transferred with a sterile needle to cooled poured plates of a suitable medium. When a rotted tuber showed a noticeable difference, in color or otherwise, in different regions of the decayed part, a separate set of plantings was made from each region. The actual isolation of more than one Fusarium from a single tuber shows that this precaution was, at least in some cases, worth while.

Several days after plantings were made, if any fungi were present they usually had made considerable growth and often allowed a preliminary macro- and microscopic comparison of the isolated organism with any others of the same series of isolations or of former ones.

In case a fungus thus obtained was different in some way[6] from the others, two transfers into test tubes of a suitable medium were made for further study. In order to make a culture from the start as pure as possible, these transfers were made from the very margin of the colony. But if in a colony there were apparently two or more organisms growing together, this method would invariably lead to isolation of the rapidly growing one and loss of the slower-growing one. In order to save the latter also, the plates with original plantings were kept for a long time after the first transfers were made, and now and then dilutions were made from the old colonies. In the majority of cases this work was useless, but in two cases there were isolated very slow-growing fungi — *F. dimerum* var. *Solani* and *F. udum* var. *Solani* — which otherwise would have been missed.

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[5] Small instruments and glassware may be sterilized conveniently by storing them in a jar of 80-per-cent alcohol. When ready to use the excess alcohol is burned off by passing the instruments through a flame. When so treated the instruments are sterile, perfectly dry, and not too hot for immediate use.

[6] Rate and character of growth, color, and type of spores were at first almost the only characters on which these organisms were judged.

The cultures obtained by transfers from original plantings seemed to be, and usually were, pure cultures from the start. Nevertheless it was evident that some method of obtaining cultures from single spores must be employed before a comparative study of the organism could be profitably begun.

In those few instances in which a culture did not produce any spores for a long time and which appeared to be a mixture of more than one fungus, an attempt was made to separate the organisms by the mere planting of a small bit of the fungous growth in the center of a newly poured plate. In only one case was the result satisfactory. This was when a bit of mixed growth of *F. arcuosporum* and *Ramularia Magnusiana*, on being planted in a plate, produced from the start on one side a pure growth of one fungus and on the other side a pure growth of the other fungus. In all the other cases a culture, if transferred into several different media, sooner or later always produced a sufficient number of spores; and in order to obtain a pure culture the poured-plate method of dilution was invariably employed.

Considerable economy of time and labor was effected by placing a number of separate drops of sterile water in a sterile plate. By transferring spores from drop to drop, a drop is soon secured in which the number of spores is such that a small loopful transferred to a drop in another plate will contain only thirty or forty spores. The melted and properly cooled medium is then poured into the plate, and distribution of spores is effected by giving the plate a rotary motion before the medium has hardened.

The first observation of the dilution plates was made about a day later. At this time most of the spores had germinated and could be observed from the bottom of the plate with comparative ease. When a single spore at some distance from the others was located, it was marked with a circle of india ink. In this manner several spores were marked and the plate was left for another day, after which the colonies were usually large enough to be observed with the unaided eye. Transfers could then be made easily and surely. These transfers have been kept as the stock cultures. The dilution plates from which stock cultures were obtained were kept for several weeks longer, in order to see whether all the colonies were alike.

In only three cases, after the first dilution, did there appear to be different colonies present in the same separation plates. In one of these cases this was the result of a mixed growth of two distinct fungi. It is of some interest to note here that the mixed culture appeared to be a fine specimen of a pink fungus. On dilution it gave rise to a brick red fungus, *F. metacroum*, and a white one, *F. diversisporum*. In the other two cases the fungi obtained from the differently appearing colonies represented most closely related organisms which never were isolated again, but which nevertheless remain distinct from each other. Whether they represent mutations, fluctuations, or mere chance coexistence of the two actually different organisms, is a matter yet undecided. They are tentatively designated as two different varieties — *F. bullatum* var. *roseum* and *F. arthrosporioides* var. *asporotrichius.*

The stock cultures were subsequently repeatedly rediluted, and, as was to be expected, almost invariably[7] every dilution plate showed all the colonies from the same stock culture to be alike.

As it is not possible to use a high-power objective with the ordinary petri dish, and as single spores of some of the species are very difficult to locate because of their size and lack of color, there was always the possibility that some of the cultures which seemed to be pure were really mixtures, for it is a well-known fact that the spores of many fungi often cling together tenaciously. In order to make absolutely sure that pure cultures were being dealt with, the further precaution was observed in 1913 of locating single isolated spores with a high-power objective and securing cultures from them.[8] The cultures thus obtained were in all respects similar to the original stock cultures, thus confirming the reliability of the poured-plate dilution.

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[7] Only two exceptions were observed: (1) In the case of *F. metacroum* var. *minus* among characteristic red colonies, there appeared one colorless colony. For a while the absence of color was a constant character, but later the color was produced again. (2) In the case of *F. diversisporum* among colonies with high aërial mycelium there was a colony almost without aërial mycelium. In subsequent generations the aërial mycelium reappeared almost to the same extent as originally.

CULTURE MEDIA

It is a well-known fact among mycologists that under different environmental conditions many fungi vary considerably in their macro- and microscopic characters. One of the most important factors in variability is the substratum. According to Thom (1910), certain characters in Penicillia appear only when the fungi are grown on a certain medium. Other instances of a similar nature might be cited.

Because of this variability of many fungi with variation in the substratum, it seemed indispensable in this study of Fusaria to use a number of different media in order to find the extent of the variability in the Fusaria and to determine, if possible, which media could be most profitably employed in this kind of work.

The so-called " natural " media, as well as artificial media, were used.[9] Those employed most extensively were potato tuber and stem plugs, and hard agars of potato, lima bean, and oat. For the study of color production, from 8 to 10 per cent of sugar (glucose) was added to one of the above agars, usually to potato agar. In all other cases the agars were used without glucose or with a small amount of it (0.5 per cent). In a few instances the agars were more or less acidified by the addition of small quantities of lactic or citric acid (from 1 to 3 drops of 50-per-cent acid to 10 cubic centimeters of the medium); otherwise they were used as they were after the process of cooking and sterilizing.[10]

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[8] The method employed was as follows: A number of drops (from eight to ten) of sterilized potato broth were placed on a sterile glass slide. Dilution transfers were made from drop to drop until such a dilution was secured that on removing a small droplet on the flattened end of a platinum needle it was found by microscopic examination that in many cases a single spore could be obtained. From such a drop nine transfers were made to a sterile, but somewhat greasy, cover glass. By placing the cover glass over a tubular glass cell or a van Tieghem cell, each individual droplet could be examined with a high-power objective. Droplets containing no spore or more than one spore were wiped off at once with a pointed piece of blotting paper. In thus removing spore droplets, the spores also were invariably removed. Sterile water was placed in the bottom of the cell and the cover glass was sealed to the cell with sterile water. The cells were then placed in a moist chamber for about twelve hours, and by this time the spores had usually germinated and could be observed with much greater ease. This observation was almost indispensable in the case of those species that produce numerous minute microconidia, as any such could be easily detected at this stage. Only two or three droplets bearing single unmistakably germinating spores were allowed to remain on the covers. At the end of twenty-four hours more, the growth of mycelium from the single spores was usually sufficient to be seen with the unaided eye and could be transferred readily with a finely pointed needle to a suitable medium.

[9] The media used were: (1) Natural — potato, bean, and pea stems; rye straw; canes of red raspberry; grains of rye, wheat, oat, barley, corn, and rice; corn meal and oatmeal; whole potato tubers and plugs of potato tuber. (2) Artificial — potato, lima-bean, oat, corn, and nutrient agars (hard and soft, from 1 to 3 per cent agar, neutral and more or less acidified, without and with different amounts of glucose).

The method of preparing all natural media was very simple. It consisted in cutting suitable pieces, tubing, and sterilizing in an autoclave for twenty minutes at fifteen pounds pressure. Enough distilled water was added to the tubes to keep the cultures in good growing condition for from three to four weeks, the exact amount, of course, depending on the size and succulence of the material used. The decoctions that were most commonly used were prepared in the following ways:

Potato decoction.— Two hundred grams of peeled potato tubers were sliced, 1000 cubic centimeters of distilled water was added, and the material was cooked for about forty minutes in a double boiler. The clear liquid was then decanted and the volume restored.

Lima-bean and oat decoctions were prepared by soaking 100 grams of ground lima beans or oats in 1000 cubic centimeters of distilled water at 60° C., in an incubator for an hour. The liquid was separated by straining through cheesecloth and the volume was restored to the original 1000 cubic centimeters.

Corn meal decoction was prepared as described by Shear and Wood (1913); that is, from about 35 to 40 grams of corn meal were treated in the same way as 100 grams of lima beans or oats in the preceding case.

For the artificial media, to 1000 cubic centimeters of a decoction were added agar (10, 15, or 30 grams, these amounts corresponding respectively to soft, medium, and hard agar) and glucose (0 to 100 grams), and the whole was cooked in a double boiler over a free flame for an hour, or, more exactly, until all the agar was well dissolved. The liquid was then tubed, plugged, and sterilized in the same way as were the natural media.

At the present stage of this work, it seems that all the labor spent on using so many different media was more or less wasted. It seems that the same results could as well be obtained with but a few good media, preferably the following: a hard oat agar (without glucose), a stem and tuber plug, and a potato agar with about 5 per cent of glucose. All other media used in the course of this work did not prove to be of any specific value, and at the best gave the same results that were obtained with those just named.

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[10] When an acid was added to an agar this was done after the medium was sterilized, the medium then being quickly cooled. This quick cooling was necessary because otherwise an acidified agar often would not solidify as well as it should.

For determination of the color of the substratum and also for study of the colony growth, it would perhaps be best to use poured plates with about 10 cubic centimeters of a clear agar. In this work potato hard agar with from 5 to 10 per cent of glucose was almost exclusively used for this purpose. For the study of other characters, in most cases cultures were made in common test tubes.

EFFECT OF VARIOUS MEDIA ON DIFFERENT CHARACTERS OF FUSARIA

In the course of this work it was observed, in partial confirmation of the statement by Appel and Wollenweber (1910: 12–23), that certain media affect fungous growth more or less characteristically. A medium too rich in nutrients, especially in glucose, usually gives cultures with more or less abnormal spores, the abnormality showing itself in a too dense granulation of the protoplasm, in more or less considerable swelling of the spore cells, and often in abnormal septation, size, and shape.

Media rich in glucose usually increase the density of color produced by these fungi, and often also change its character. For example, a pink fungus, *F. arcuosporum*, is turned to a clay-colored one; or the fungus *F. angustum*, which is colorless or nearly so, is turned to a more or less bright purplish-vinaceous one; and so forth.

Excess of water in a medium usually leads to comparatively quick degeneration (self-digestion?) of the spores, and, in general, to a shortened duration of the vitality of the culture. Its presence, at least in case of a soft agar as compared with a hard agar, is usually unfavorable for the normal development of aërial mycelium.

A medium comparatively poor in nutrients, such as corn meal agar, seldom gives rise to sclerotia and plectenchymic sporodochia; but in a way it is a good medium for the study of chlamydospores, which are produced here more or less freely and stand out more clearly than in other media.

Whole potato tubers (steamed) often are most favorable for production of large sporodochia; this medium, and also potato tuber plugs (also steamed), show the largest sclerotia.[11]

An agar, especially such a one as oat hard agar, often gives all the forms of fructification for these fungi, with "normal" spores and more or less typical and brilliant color production.[12]

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[11] See also Wollenweber (1913 a: 25).

On the plugs made from stems of different plants, spore production seems to be normal.[13] The spores are of typical and comparatively uniform shape, septation, and size, without too dense granulation of the protoplasm, and with a long duration of vitality. It often is found, however, that on such media spores are less normal than, for instance, on a hard agar.

Presence of the epidermis on the stem and tuber plugs seems to favor production of fewer but better developed sporodochia,[14] and often lessens development of aërial mycelium.

Certain media, such as boiled rice, give a color that is typical for certain related Fusaria.

EFFECT OF LIGHT AND OF REACTION OF THE MEDIUM ON DIFFERENT CHARACTERS OF FUSARIA

As was stated in the introduction, very little systematic work has been done on the ecology of the Fusaria. The few things which have been found by other workers and by the writer, and which have some value in the determination of these fungi, may be summed up briefly as follows:

1. A diffuse daylight may affect color production considerably (Smith and Swingle, 1904:48–49), but in most cases the effect is only slight or there is almost none. It usually intensifies the colors produced by these fungi. A very strong light often makes the colors somewhat duller, especially in the case of Fusaria producing bright red colors, these being turned toward brown hues. No noticeable effect of the light was observed on other characters of these fungi, although Appel and Wollenweber are of the opinion that the spores are of a more normal type in the light than in the dark.

2. According to Appel and Wollenweber the reaction of the medium has an especially noticeable effect on blue colors, which can appear only in a medium of neutral or rather alkaline reaction. The blue color in a medium of an acid reaction will appear as orange. True red colors remain

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[12] This observation is apparently in some contradiction to the observations of Appel and Wollenweber (1910:12–13), but indeed it is not so; because, judging by the "artificial" media actually used by them, their observation of unfitness of such media for study of "normal" growth of the Fusaria was based on "soft" agars too rich in sugar. The writer also found that such agars produced abnormal growth.

[13] This was observed first by Appel and Wollenweber (1910), and on this observation mainly they concluded that the only way to study the Fusaria properly is to study them on such "natural" media.

[14] This was first observed by Appel and Wollenweber (1910).

red even in a medium of an alkaline reaction. The observations of the writer, so far as they go, are in full agreement with the above statements; to which it may be added that certain fungi which in a neutral medium produce a grayish white aërial mycelium (*F. sclerotioides*), in the same medium strongly acidified (0.4 per cent by weight of citric acid) produce an aërial mycelium of a pink-vinaceous shade.[15]

It was observed also that acidity of the medium lowers the rate of the fungous growth and makes zonation of the colony more prominent and closer. The retardation of the growth depends on the kind of acid (see also Smith and Swingle, 1904:42, 48) and its concentration; different Fusaria are not affected in the same degree, some tolerating more acid than others (see also Lewis, 1913:238).

THE GENUS FUSARIUM

Appel and Wollenweber (1910:4–12, 23–61) present a very detailed, critical study of the genus, with the following list of synonyms:

Atractium Link pr. p. (1809)
Fusidium Link pr. p. (1816 and 1825)
Fusisporium Link (1824)
Selenosporium Corda (1837)
Fusoma Corda (1837)
Pionnotes Fries (1849)

The description of the genus Fusarium given by these investigators, when other data presented by them are also taken into consideration and using the terminology of Lindau (1905, 1908–1909), may be stated in brief as follows:

Hyphomycetes of Mucedinaceæ-Hyalophragmiæ, Hyalostilbaceæ-Phragmosporæ, and Tuberculariaceæ-Mucedineæ-Phragmosporæ groups, with smooth, not appendiculate, mostly sickle-shaped, acrogenous, noncatenulate conidia.

The genus as delimited by these authors was to include also forms such as *F. dydimum* and *F. Willkommi*, with cylindrical, one-septate spores, and bacilliform, one- to five-septate spores.

The writer has not sufficient material on hand to warrant any change in the above characterization of the genus; but in the course of this work, a true Fusarium never was observed which would produce a typical coremium[16] or a typical pionnotes,[17] that is, the two fruiting forms on the basis of which the genera Atractium and Pionnotes were founded by their authors and which are now reduced to synonymy by Appel and Wollenweber because these investigators find in the Fusaria certain structures resembling these two forms.

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[15] The medium used in the instance cited was potato hard agar plus 5 per cent of glucose. The cultures were made in petri dishes.

That one of these forms, Pionnotes, is a distinct form, and that its characters can be used as a basis in a classification of Fusarium-like organisms, has been shown lately by Wollenweber. After citing *Fusarium* (*Pionnotes*) *udum* and its variety as instances of the Fusaria without "Fusszelle," he (1913 c: 206) says: "Beiläufig bemerkt, bilden solche Fusarien zusammen mit *F. aquaeductum* eine gute Section der Gattung, die ich Eupionnotes nenne wegen des Übergewrichtes dieser Sporenverlagerung."

There is very little doubt that a true coremial form of fructification is quite distinct, and does not occur in the species of Fusarium observed by the writer.

With respect to the present status of the genus Fusarium, some recent changes must be considered here. Wollenweber (1913 a:33) transferred from the genus Fusarium to the genus Ramularia all forms with conidia of *F. dydimum* type when chlamydospores are present.[18] The same author (1913 c:225), somewhat later, also excluded from the genus Fusarium forms with conidia of the type of *F. Willkommi* and transferred them into a new genus, Cylindrocarpon,[19] established for this purpose. Thus, in fact, all forms having cylindrical conidia or conidia with rounded ends are excluded by Wollenweber (1913 c:239) from the genus Fusarium. The genus Sepedonium Link (1809), according to Wollenweber (1913 c: 200), is only a chlamydosporial stage of *Fusarium orthoceras*.

It appears, then, from the study of the Fusarium-like organisms of potatoes (which, on the whole, represent a great diversity of forms) and also from a careful survey of the genus Fusarium as it stands in literature, that the following characters are of generic value:

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[16] The terminology which is used here is the same as that of Lindau.

[17] Only one Fusarium-like organism was isolated from potatoes which produces a fruiting layer very similar to a pionnotes (*F. udum* var. *Solani*), but it is very distinct from all the other Fusaria and can be used rather to support than to disprove the above statement.

[18] In those cases in which chlamydospores are not present, Wollenweber says the fungi have a perithecial stage of the genus Mycosphærella.

[19] This genus is to include only those forms for which a perithecial stage has not yet been found. *F. Willkommi* has been connected with *Nectria galligena*.

1. Color of conidia and mycelium, never of a plain gray or black color but mostly of various brilliant hues.

2. Conidia dorsiventral,[20] attenuate, pedicellate,[21] not appendiculate, smooth, normally not constricted at the septa, distinctly three- (or more) septate,[22] acrogenous, not in chains.[23]

3. Conidiophores with single to irregularly whorled branches, never truly dichotomous nor of a strictly penicillate or verticillate type. The conidiophores, through many times repeated branching or also by growing side by side with other conidiophores, typically give rise to macroscopically observable, dense tufts of conidiophores covered more or less deeply with a somewhat slimy mass of spores. Such fruiting bodies, tuberculate in form (sporodochia),[24] may be with or without a plectenchymic (=pseudoparenchymic), flat or wartlike base, without any differentiated enclosure.

4. Chlamydospores (endogenous, double-walled, resting bodies) terminal and intercalary, or only intercalary,[25] or none, and produced both by mycelium and by spores.

5. Mycelium composed of hyphæ which are always distinctly, but not closely, septate, and irregularly, never dichotomously, branched, the secondary branches usually thinner than the primary ones; protoplasmic content of the mycelium for the greater part plainly present and usually distinctly vacuolate. The rate of growth in artificial media, when compared with the Fusarium-like organisms studied, is comparatively high.

It must be added here, as a general remark to the characteristics of the genus Fusarium given above, that in this case, as well as in any other attempts at classification of natural phenomena, the boundaries laid down for separation of one group of phenomena from all the rest have only a relative value. Thus an organism may deviate[26] in a greater or less degree in one or a few of the characters given above, and yet, so to speak, be a good Fusarium provided all other characters of this genus are well expressed by the organism.

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[20] This term is used by Appel and Wollenweber (1910).

[21] This term is used by Wollenweber (1913 c). See his key on page 219 of reference cited.

[22] Fusaria-like organisms with one-septate conidia are rare. Two such organisms were isolated from potatoes, and from over one hundred and sixty Fusaria recorded by Lindau (1908–1909 : 517–588) only seven are definitely stated to have one-septate conidia; and of these seven at least two are undoubtedly not Fusaria if the changes made in this genus by Wollenweber are considered.

[23] Microconidia may be produced in chains. This is true in case of certain Fusaria of corn and of coniferous seedlings. (See Sheldon, J. L. A corn mold [Fusarium moniliforme n. sp.]. Nebraska Agr. Exp. Sta. Rept. 17: 23–32, fig. 1. 1904.)

[24] The terminology used here is the same as that used by Wollenweber (1913 a : 24, footnote).

[25] The only Fusarium-like fungus isolated by the writer which has only terminal chlamydospores seems not to be a typical Fusarium. (See *F. cuneiforme*, key and description.)

[26] Of course a true Fusarium, evidently, in no case can be of a plain gray or black color (in mycelium and conidia as well), or have non-septate or cylindrical macroconidia with both ends rounded. (In regard to the macro- and microconidia, see page 116.) Its conidia cannot be appendiculate nor its conidiophores of a true verticillate or any other specifically peculiar type.

VARIABILITY IN THE FUSARIA

Many of the morphological and physiological characters of the Fusaria show marked variability, and at first seem to be of such a nature as to discourage any attempt to treat these fungi on a morphological basis. Thus Smith and Swingle (1904:27), in regard to their *F. oxysporum*, say: "......This fungus showed a number of very striking variations. For this reason it is impossible to give a general description that will hold universally." And after quoting descriptions of eleven old species of Fusarium from potatoes, the authors conclude as follows (1904:51): "Judged by the above descriptions, we have had a half dozen or more species of *Fusarium* in our culture tubes, some of them 'new species,' and yet all were the product of a single spore. This does not mean that there have been in our cultures any very wonderful transmutations of one thing into another, but only that organisms respond to their environment, and that 'species descriptions' of the kind cited have not taken this fact into consideration, and consequently are worthless for scientific purposes. This is not a new idea, but it is a fact to which the attention of systematic mycologists might be directed profitably at frequent intervals."

Sometimes variability, for example in the type of conidia, is so great that a student gives up hope of determining the actual type. Thus Wilcox, Link, and Pool (1913:24) conclude their discussion on the form of the conidia produced by their fungus[27] as follows: "All sorts of stages are shown in the plate, so that each one can judge for himself, as it is possible that one who has studied a great number of species of *Fusarium* will be able to pick out the characteristic form which can be set aside for this particular species."

A great number of instances of variability in this group also could be cited easily from the species presented in this paper. But it will suffice to state here only those instances of variability which are, perhaps, most important and more or less common:

1. The type of the conidium varies in many Fusaria from micro- to

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[27] *F. tuberivorum* Wilcox and Link, which according to Wollenweber (1913 c : 206) is identical with *F. trichothecioides* Wr.

macroconidia, and both sometimes are of a very diverse type even within themselves. (See *F. diversisporum.*)

2. Conidiophores often vary from mere minute projections on the side of a hyphal thread to a complex dendroid structure often of a size observable macroscopically.

3. In the Fusaria for which production of sporodochia, plectenchymic stromata, or sclerotia is typical, it sometimes happens that they are not produced even under apparently favorable conditions. These structures may vary in size, number, and form.

4. The presence or absence of color is an especially variable character. Changes in type of color may occur, but such changes are only apparent and in all known cases can be explained on the basis of the difference in the reaction of the medium (certain orange-red colors in acid become blue in an alkaline medium), or, when a typical color is a compound one, the change may be due to the preponderance of one or another of its elementary colors.

5. Extent of the development of aërial mycelium, zonation, rate of growth, and character of the margin of the colony, may also vary to a considerable extent.

6. Especially great variation may take place in the relative production of the different types of conidia and chlamydospores.

It might appear at first glance that the variation of these fungi is so great as to leave no firm ground for morphological treatment of the group. And yet the actual situation is far from being so hopeless. First of all, the great majority of the variations mentioned above occur, as did those observed by Smith and Swingle (1904:59), under different environmental conditions; while the variability under identical conditions usually (see also Lewis 1913:225) is very slight. Secondly, the cases of variability cited above are primarily cases of variation between presence and absence of certain characters — which is, after all, of no great importance, because when a certain character is present it is always peculiar to a specific type of organism and thus this organism can be separated from others. Then there are certain Fusaria which under almost any but extreme environmental condition remain nearly the same throughout (as *F. udum* var. *Solani* and *F. cuneiforme*). And finally, the most important character in the classification of these fungi — the type and the shape of the conidia

— is after all sufficiently stable to be used safely in a morphological treatment. Even the size of conidia, when a sufficient number of measurements is made and averaged and when only conidia of the same type are compared, is of rather surprising uniformity and stability. It is believed that every one will agree with these statements after an examination of the data presented here in the descriptions of species.

That Fusaria can be separated on a strictly morphological basis was first definitely demonstrated by Appel and Wollenweber.

RELATIVE TAXONOMIC IMPORTANCE OF DIFFERENT CHARACTERS

Usually the particular organism and the prominence and stability of a given character determines the relative importance of characters in classification. Thus, in certain cases septation of conidia may be considered as the important character. For instance, in the case of *F. dimerum* and *F. affine*, this character distinguishes these two fungi at once from all the other Fusaria, while in most other cases it is not of such importance. Presence of a continuous slimy layer of spores is the important character in the case of *F. udum* var. *Solani* where this type of fructification is especially prominent. Type of microconidia, when they are of typical occurrence and especially when they are of a peculiar type, as in the case of *F. sporotrichioides*, is also a most important character. Type of chlamydospores is an important character in general for dividing the whole genus into sections, but it usually has no specific value; and yet there is a fungus, *F. cuneiforme*, which can be set off from all the rest because of its terminal unicellular chlamydospores. Color may vary considerably, but on the whole its type is stable enough to be of considerable help in dividing Fusaria into sections, and sharp contrasts in color can often be used for specific differentiation.

In general the most useful and evidently sure basis for a natural classification of this group is the shape of the macroconidia.[28] Their dorsiventrality, the form of their apex and their basal cell, and their septation, and also their size, when properly used, are of considerable service in separation of species. The actual working value of each character can be seen from the keys.

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[28] Appel and Wollenweber (1910 : 34) and Wollenweber (1913 a : 26) came to this conclusion long before the writer. See also Lewis (1913 : 225).

FORMS OF FRUCTIFICATION IN PURE CULTURES

Spores

The spores in the genus Fusarium are of two kinds: conidia, or acrogenous spores, and chlamydospores, or endogenous resting bodies. The conidia in turn may be divided into two more or less distinct types: (1) macroconidia, sickle-shaped, three- or more septate spores; and (2) microconidia, oval, non- or only one- or two-septate spores.

Before the appearance of Appel and Wollenweber's work (1910) the existence of micro- and macroconidia as two distinct types was more or less generally accepted, but in that work it was concluded (page 29) that there is only one type of conidia. The latter view is, of course, correct so far as the genetic relationship between micro- and macroconidia goes. Nevertheless, the typical presence of microconidia in certain closely related Fusaria and their typical absence in others is characteristic. Besides, whenever they are present they have a comparatively constant and often peculiar type of their own. Thus, they can be used as a good natural basis for classification of these fungi, and for that reason must be considered as a type separate from macroconidia.

In this paper the term *microconidia* is applied to all nonseptate, and seldom to one- and even to two- or three-septate, conidia of a different shape from that of the macroconidia, which are sickle-shaped and usually three- or more septate. Different forms of macroconidia are shown in figure 1 (page 112).

Chlamydospores may be borne on the ends of special lateral branches of the mycelium (terminal chlamydospores), or they may be intercalary. They often are produced in the ends of the conidiophores, in the conidia or in the ends of special branches from the conidia. The chlamydospores are single, in short to long chains or in more or less large clusters. They are of common, though not of general, occurrence, and in a number of Fusaria both terminal and intercalary chlamydospores are present; in some others there are only terminal, and in still others only intercalary chlamydospores have been observed. A number of Fusaria evidently have no true chlamydospores; they may possess structures with dense content, but these structures are not thick, double-walled bodies.

Forms of fructification

Sometimes, as in the case of the conidia on aërial mycelium of *F. trichothecioides*, and also in the sporotrichial form of *F. sporotrichioides*, the conidia are borne singly and remain thus in the form of a powder. Usually, however, they adhere to the tips of conidiophores for a time, forming balls of variable size. This may be the case with microconidia as well as with macroconidia. In certain Fusaria, such as *F. cuneiforme*, and in all members of the section Martiella, these balls of conidia are especially prominent; but they are very common also in the other Fusaria and therefore cannot be used for specific differentiation.

When there is no room for old conidia to be pushed aside — that is, when the fruiting branches are very numerous and close together — a considerable and continuous mass of spores results; these, when the air is moist, form a roundish, wartlike heap of spores, which, with the conidiophores producing them, is known as a **sporodochium.** When the air is comparatively dry, the spores are pushed up in more or less curled, long, tendril-like columns. This is often observed especially in many cultures on sterilized canes of red raspberry and in stem plugs of other plants.

Often small sporodochia are produced on and strewn all over the aërial mycelium, producing a picture very characteristic for *F. subulatum* and some other Fusaria. In other cases minute and numerous sporodochia are produced very close to or on the surface of the substratum, and when these minute sporodochia are very numerous they form a nearly continuous, slimy layer of conidia. The fructification then resembles a pionnotes and is called here **pseudopionnotes.** In one case a seemingly true pionnotes[29] — that is, a thick, continuous, slimy layer of spores — was observed (in *F. udum* var. *Solani*). A pseudopionnotes may be produced under aërial mycelium which may more or less mask its macroscopic appearance, as in the case of several Fusaria of the section Elegans; but in certain cases there is no aërial mycelium over it, and the pseudopionnotes remains fully exposed and characteristic for certain species. (See *F. metacroum*, *F. falcatum*, and *F. discolor* var. *sulphureum*.)

Mycelial threads in many Fusaria often run parallel and anastomose more or less closely, thus producing a ropelike structure which may come up into the air in an irregular fashion and which also may bear more or less abundant conidia produced on side branches. These ropelike structures then resemble coremia, which are columnar fruiting bodies, typical of the family Stilbaceæ. No true coremia were observed in any of the Fusaria presented here.

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[29] The definition is taken from Lindau (1908–1909: 509).

A stroma, in the sense employed here,[30] is the fungous layer on which aërial structures (aërial mycelium, conidiophores, and spores) are produced. The stroma may consist of more or less loose hyphæ, or it may form a dense pseudoparenchymic (plectenchymic), continuous sheet, fleck, or prominent wart. There are often produced also roundish, more or less wrinkled, often shiny, bodies, resembling true sclerotia. They are of a dark blue color, and among the Fusaria studied in the course of this work they were observed only in certain species of the section Elegans; though according to Wollenweber (1913 a:32) they are very common also in the section Roseum, and are characteristic for one species, *F. sclerotium* Wr., of the section Gibbosum.

METHOD OF STUDY AND PRESENTATION

The method actually employed in this work consisted in the cultivation of pedigreed strains of the various organisms on various natural and artificial media. Almost without exception, all the strains were transferred to a new medium on the same day and the whole set of cultures was kept under the same environmental conditions. In all important cases duplicate cultures were employed. For the inoculum, as far as possible, similar material was used — that is, only mature spores or only aërial or only submerged mycelium. The importance of the same environmental conditions for a comparative study is evident. It applies equally as well to the kind of inocula used, as it was found[31] that often an inoculation made with spores tends to better spore production, and an inoculation with mycelium often results mainly, at any rate at first, in the production of mycelium.

In order to bring all cultures to the same stage of maturity and also to assure their purity, dilutions in poured plates were made again and again for the entire set of the Fusaria (of isolations made by the writer, as well as of the organisms obtained in culture from other sources), and then new transfers were made from colonies about two days old produced in the plates.

In the macroscopical examination of the culture, special attention was given to the presence or the absence, and the character, of aërial mycelium; to the kind of fructification layer (pionnotes-like, sporodochial, and so forth); to the color of spores, of aërial and submerged mycelium, and of substratum; and to the production of special structures such as plectenchymic stromata and sclerotia.

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[30] A slightly different definition of stroma is given by Wollenweber (1913 a: 24, footnote).

[31] See Appel and Wollenweber (1910: 13), and also Lewis (1913: 209). The same was frequently found to be the case also in the course of this work.

In the microscopical study, the different types of conidia, chlamydospores, and conidiophores received special attention. The observations were recorded by means of camera lucida drawings[32] and necessary measurements.

When measurements were of any importance,[33] ten conidia (or chlamydospores) were measured and only the average and the extremes of these measurements were recorded. In cases of special variability of the material, records were made of fifteen or twenty spores of each important type of septation and shape.

In making camera lucida drawings, care was taken to picture the apical and the basal ends of the conidia with the utmost accuracy; every kind of conidium occurring in a culture was drawn, but the typical and the exceptional cases, as they appeared, were marked off.

As a rule, measurements and drawings usually were made of the conidia taken from sporodochial or pionnotes-like masses of them, because such conidia on the whole are more uniform and typical for a given organism. Here it should be noted that for the measurements and drawings it is highly important, at least in case of very closely related organisms, to use material analogous in all respects — age, type of fructification, and environmental condition.

The fungi were studied, not only on different media, but also at different stages of their growth. The latter factor is almost as important as the former, because in certain cases, as in *F. angustum*, conidia in the best condition (most regular, and so forth) were observed when the cultures were very young. Some characters, however, become manifest only after a culture has reached a certain stage of maturity. This is often the case with chlamydospores and color production. Sclerotia often appear in comparatively old cultures and continue to grow for some time.

The presentation of the species of Fusarium is based on the following main principles:

All drawings (with only a few exceptions) are intentionally made to the same scale as those of Appel and Wollenweber — thus far the one fundamental work on this subject[34] — so that comparison can be made easily. The drawings represent all types of conidia, abnormal ones included, so that no one may be misled by types actually observed by him and by those given here. This might easily be the case if only normal material were presented, because our understanding of what is normal is very relative, is often too broad, and seldom corresponds to the things as they are. The particular forms that seemed to be normal are indicated by the drawings in figure 1 (page 122).

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[32] All drawings were made from living material mounted in water, and, with a few exceptions, with an oil immersion lens. In order to prevent movement of the spores in water, it is necessary first to use just enough water to keep air from underneath the cover glass, and then to spread around the cover glass some oily substance, as cedar oil, which does not dissolve in water and which does not dry out quickly.

[33] A number of fields should be examined before measuring in order to see the prevailing type, and then measurements of the conidia of the prevailing type should be taken. This leaves much to personal interpretation, but otherwise it would be necessary to take many more measurements.

Usually no attempt was made to represent the structure of the cell content, because it was not considered of taxonomic value. In those few cases in which the structure of the plasma seemed to be characteristic it is shown in a very approximate way.

The microscopical character of the mycelium, so far as observed by the writer, cannot be used for specific differentiation of these fungi; therefore no attention has been given it, either in drawings or in measurements, except in a very few instances when certain striking peculiarities have been so recorded (Figs. 34M and 43L).

In giving measurements of the size of spores, it was considered necessary, first, to give an average size for conidia from a particular culture, as well as the average of the measurements for the entire series of cultures. Thus the measurements have a definite meaning and are comparable with one another, provided the measurements are taken separately for each type of spore.

In arranging the species according to their relationship it was found convenient to follow Wollenweber's example (1913 a:26–27), and divide all the Fusaria into sections. Most of the sections are the same as those of Wollenweber, but because of some organisms presented here that could not be placed in any of his sections, certain new sections have been established provisionally.

In connection with taxonomic study and presentation of the Fusaria, it is necessary to discuss, at least briefly, the conception of the so-called normal culture. The observations of the writer in this regard are principally of the same nature as those of Appel and Wollenweber (1910:21–22) and can be summarized as follows:

1. A culture can be in the state of undevelopment (*Ankultur*) when the growth shows mycelium to the entire exclusion of, or to an abnormally

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[34] It is firmly believed that standardization of the subject presented is especially important in the study of this difficult group of fungi.

poor production of, spores; other forms normally present in the culture may also be absent. This state of culture may sometimes exist when a fungus is transferred from a mycelial growth, especially when it is taken directly from host tissues.

2. A culture is considered to be a normal one (*Normkultur*) when all forms typical to the fungus — and especially the most important form, the macroconidia — are abundant, comparatively uniform in size and shape, smooth in outline, and so forth.

3. After a long cultivation on artificial media, a fungus may lose certain characters, such as ability to produce certain color and also its virulence as a parasite. Such a state of culture is that of degeneration (*Abkultur*), and it may be accompanied also by smaller size and abnormal septation of the conidia. (Only loss of color was observed by the writer; the other observations are those of Appel and Wollenweber, 1910:22.)

4. In the first period of growth of any one of the Fusaria, the first conidia produced usually soon begin to produce new conidia, sometimes on minute papillæ located directly on the conidial walls, and sometimes on more or less well-differentiated and well-developed conidiophores, the process often much resembling the budding process in yeasts. In such young cultures the conidia are more or less swollen, their contents are commonly densely granular, and septation is not clear. This period (*Jungkultur*) may last from one to five or more days.

5. After the period just described, the fungus, when in normal condition, produces conidia which remain for a greater or a less length of time, of perfectly smooth outline and with clear septation. At this state of maturity (*Hochkultur*) the conidia are also of most uniform and typical shape and size, and it is the important stage for taxonomic study of these organisms.

6. Finally there comes a period (*Altkultur*) in which the conidia begin to disintegrate through the process of self-digestion, or at least become of less uniform and perfect type, and after a period of time the culture begins to lose its vitality.

These variations in character of the cultures show that only normal and mature cultures must be considered, though the character of other conditions may also give some help in identification. Another point which must be clear from the account given above is that in the study of these fungi they must be grown for a long period of time, under different cultural conditions and from different kinds of material for inocula. Then, after observation of all the stages, it will be possible to pick out easily the typical and normal one and base judgment mainly on that. Most minute attention must be paid to the shape of macroconidia.

In order to avoid much of the possible confusion in regard to the color production by the fungi, it will perhaps be not out of place to mention again that a convenient standard of colors must be used. Ridgway's (1912) color standards have been used in this work.

IDENTIFICATION OF THE FUSARIA WITH PREVIOUSLY DESCRIBED SPECIES

An identification of the Fusaria of potatoes with Fusaria formerly described as occurring on other substrata and even on potatoes, is in most cases impossible or at least rather doubtful. The species have been described mostly from material as it was collected in nature. After what has been said in the foregoing pages about variability of the Fusaria, about their common occurrence in different, often abnormal, stages, about the necessity of most minute attention to the peculiarity of the shape of the macroconidia, and about the importance of good drawings and of measurements properly made and presented, it can easily be seen what small chance there is of identifying an organism from any typical description of one of these species. The figures are often absent, and when present they are either too schematic or so inaccurate that they might just as well be omitted. Any citation in support of this statement would be superfluous.[35]

There are a number of different Fusaria which agree with a certain description and therefore might be considered as the same species. At the same time the very same organism may have other characters which, if studied alone, would surely set it off as a distinct species. An extensive illustration of this state of affairs is given by Appel and Wollenweber (1910:9–12), but the most impressive case is that of Smith and Swingle (1904:50–51). Therefore, although the literature on the subject was fully examined, especially publications of descriptions supplemented by any kind of illustrations, usually no definite conclusion in regard to identity could be arrived at. Because of this, and also because the most important literature is already listed in a few works on this subject (especially by

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[35] Appel and Wollenweber (1910:12) in this connection made the following statement: "Oft blieb uns daher weiter nichts übrig, als neue Namen zu geben und ihnen so genaue Beschreibungen beizufügen, dass nunmehr die Arten immer wieder erkannt werden können."

Appel and Wollenweber in 1910, and by Wollenweber in 1913), this literature is usually not cited and a bibliography is omitted.

The condition of the taxonomic literature on species of the genus Fusarium in general is characterized by Wollenweber (1913 a:41) as presenting "an almost hopeless confusion." The only favorable exceptions known to the writer are the works of Wollenweber, alone and in association with other authors. These works are fully considered here. In fact there is hardly any difficulty in recognizing Fusaria described by Wollenweber. Nevertheless all available cultures of his organisms were grown along with the other Fusaria, and a thorough comparison of living material was made whenever it was necessary.

When this work was actually completed there appeared the work of Lewis (1913) on certain disease-producing species of Fusarium. The work was supplemented also with Wollenweber's list of the names for the fungi studied by Lewis, and also with certain remarks by Wollenweber in regard to the taxonomy of those organisms. It appeared that certain of Lewis's organisms were the same as those isolated by the writer from potatoes. Cultures of four Fusaria, somewhat resembling certain species isolated from potatoes, were obtained by the writer through the courtesy of Dr. Morse, Plant Pathologist in the Maine Agricultural Experiment Station. On comparison these four organisms — *F. pirinum* (Fries) Sacc., *F. conglutinans* Wr., *F. citrinum* n. sp., and *F. argillaceum* (Fries) Sacc.— were found to be distinct from all Fusaria presented here.

SYSTEMATIC ARRANGEMENT OF THE SPECIES OF FUSARIUM OF POTATOES

The natural key to the species and their arrangement in sections,[36] as given here, are in the main similar to those of Appel and Wollenweber (1910:59–60) and of Wollenweber (1913 a:28–32), respectively. Some changes and additions have been made, however, in order to include many new organisms.

Variety is used as the smallest unit in the taxonomic treatment of these fungi. An organism is classed as a variety if it differs from the closely related species in only one character, even though the difference be conspicuous; the same treatment holds with respect to the organisms that differ slightly in a few characters. When the differences are slight, but in several important characters, the organisms are classed as distinct species. In general it was preferred rather to name a possibly good species as a variety than to raise a mere variety to specific rank.

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[36] The term is used by Wollenweber (1913 a: 28).

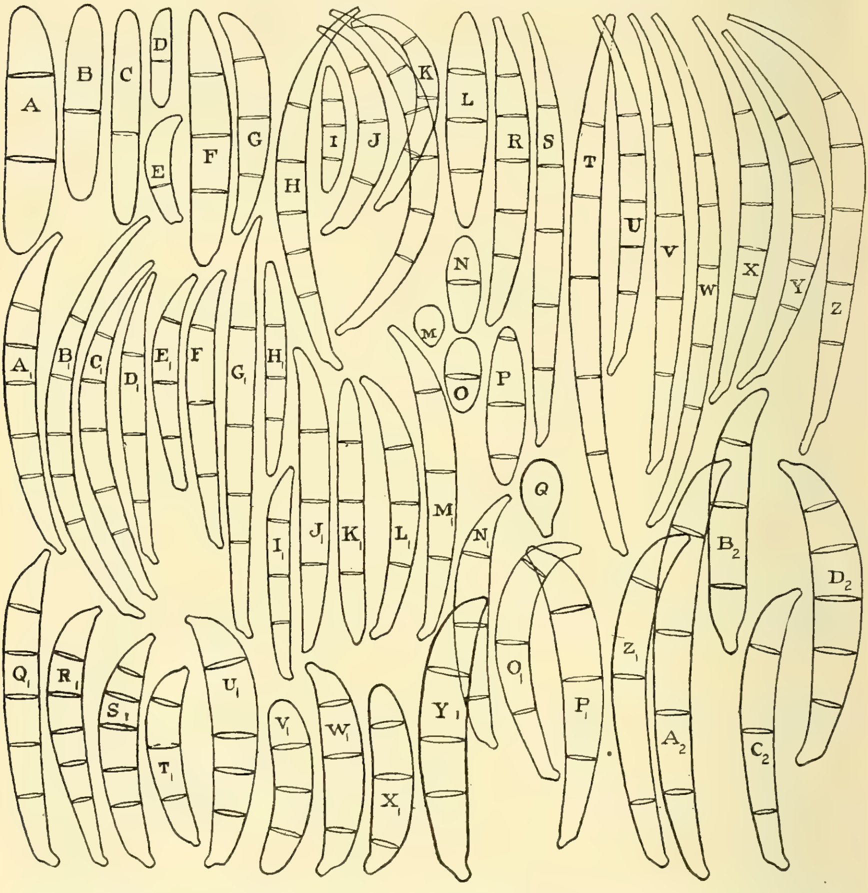

FIG. 1.— *Types of conidia of the species of Fusarium and Fusarium-like fungi found on potatoes.* A, *Ramularia Solani;* B, *R. eudidyma;* C, *R. Magnusiana;* D, *Fusarium affine;* E, *F. dimerum;* F, *F. cuneiforme;* G, *F. udum var Solani;* H, I, J, *F. ferruginosum;* K, *F. sanguineum var. pallidium;* L, M, N, O, P, *F. arthrosporioides;* Q, *F. sporotrichioides;* R, S, *F. arthrosporioides var. asporotrichius;* T, *F. anguioides;* U, *F. biforme;* V, *F. lucidum;* W, *F. subulatum;* X, *F. falcatum;* Y, *F. gibbosum;* Z, *F. caudatum var. Solani;* A_1, *F. bullatum;* B_1, *F. arcuosporum;* C_1, D_1, E_1, *F. truncatum;* F_1, *F. lutulatum;* G_1, H_1, *F. angustum;* I_1, J_1, K_1, *F. orthoceras;* L_1, *F. oxysporum var. resupinatum;* M_1, *F. oxysporum var. longius;* N_1, *F. oxysporum;* O_1, *F. sclerotioides;* P_1, *F. redolens var. Solani;* Q_1, *F. discolor;* R_1, *F. clavatum;* S_1, T_1, *F. subpallidum var. roseum;* U_1, V_1, *F. trichothecioides;* W_1, *F. discolor var. triseptatum;* X_1, Y_1, *F. Solani;* Z_1, A_2, *F. Martii;* B_2, *F. coeruleum;* C_2, *F. striatum;* D_2, *F. culmorum var. leteius. Magnification 1000 times*

DICHOTOMOUS KEY TO THE SPECIES OF FUSARIUM AND FUSARIUM-LIKE FUNGI OF POTATOES

Page

a. Conidia [37] not typically dorsiventral, apex rounded, apedicellate......RAMULARIA 264
b. Conidia typically one-septate
c. Average diameter of one-septate conidia 4.9 μ................*R. eudidyma* 264
cc. Average diameter of one-septate conidia 3.9 μ................*R. Magnusiana* 265
bb. Conidia typically two-septate..*R. Solani* 267
aa. Conidia typically dorsiventral, apex more or less attenuate, mostly pedicellate FUSARIUM 125
b. Conidia typically one-septate, three or more septa never present..Section **Dimerum** 125
c. Ventrally straight..*F. affine* 126
cc. Ventrally curved..*F. dimerum* 127
bb. Conidia typically three- or more septate
c. Dorsiventrality slight....................................Section **Ventricosum** 128
d. Chlamydospores terminal only.............................*F. cuneiforme* 129
dd. Chlamydospores terminal and intercalary...................*F. ventricosum* 128
cc. Dorsiventrality distinct
d. Conidia apedicellate, true pionnotes present...Section **Eupionnotes.** *F. udum* 131
dd. Conidia pedicellate, true pionnotes absent
e. Conidia with gradually attenuated, pointed apex
f. Conidia prominently broader in the middle, apex long and narrow, most prominently pedicellate...........................Section **Gibbosum** 133
g. Dorsally more or less hyperbolic.......................*F. gibbosum* 133
gg. Dorsally more or less elliptic
h. Aërial mycelium poorly developed, fruiting layers in form of a pseudopionnotes......................................*F falcatum* 135
hh. Aërial mycelium well developed
i. Conidia typically five-septate.............*F. falcatum* var. *fuscum* 138
ii. Conidia typically five- to seven-septate.....*F. caudatum* var. *Solani* 140
ff. Conidia of about equal diameter for a more or less considerable part of their length, apex not very long
g. Conidia typically five-septate; typical color of conidial masses red, of substratum pink to orange and brown-red; no terminal chlamydospores
h. Chlamydospores absent
i. Microconidia typically absent..................Section **Roseum** 142
j. Blue sclerotia present.........................*F. acuminatum* 142
jj. Blue sclerotia absent
k. Conidia typically in pseudopionnotes; aërial mycelium weakly developed or absent.......................*F. metacroum* 143
kk. Conidia typically in distinct sporodochia; aërial mycelium well developed
l. Sporodochia small, borne on aërial mycelium and without plectenchymic base.......................*F. subulatum* 147
ll. Sporodochia large, with plectenchymic base
m. Six-septate conidia typically present.........*F. effusum* 151
mm. Six-septate conidia typically absent
n. Five-septate conidia dominant type........*F. lucidum* 157
nn. Three- and five-septate conidia about equally present, or three-septate dominant.............*F. truncatum* 155

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[37] The term *conidia* is used here, in this key and elsewhere, in the sense of macroconidia.

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ii. Microconidia typically present, usually spindle-shaped, non- to three-septate........................Section **Arthrosporiella** 161
j. Six- to seven-septate conidia typically present, at least in pseudopionnotal stage
k. Distinct sporodochia present
l. Substratum and aërial mycelium near it typically of carmine hues, causal layer of aërial mycelium pink......*F. biforme* 166
ll. Substratum of a clay color, aërial mycelium white......... *F. diversisporum* 161
kk. No conspicuous sporodochia, fruiting layer on moist media commonly in form of a pseudopionnotes.......*F. anguioides* 169
jj. Six- to seven-septate conidia absent..........*F. arthrosporioides* 175
hh. Chlamydospores present, typically intercalary only
i. Microconidia present, pyriform.............Section **Sporotrichiella** 183
F. sporotrichioides 183
ii. Microconidia absent or not pyriform.......Section **Ferruginosum** 186
j. Typical macroconidia of about equal diameter for a considerable length, or not prominently broader in the middle, in some cases approaching the subulatum type, much curved
k. Chlamydospores typically only in mycelium
l. Chlamydospores sparse, never in masses nor in long chains.. *F. arcuosporum* 186
ll. Chlamydospores typically abundant, commonly in masses of long chains and in clusters..............*F. ferruginosum* 190
kk. Chlamydospores also in conidia or in conidia only........ *F. sanguineum* 193
jj. Macroconidia typically noticeably broader at the middle, not very pointed at the apex, not much curved......*F. bullatum* 198
gg. Conidia typically three-septate; typical color of substratum vinaceous red to purplish vinaceous; terminal and intercalary chlamydospores present......................................Section **Elegans** 202
h. Conidiophores simple or only slightly branched
i. Average macroconidia 36 μ long.....................*F. orthoceras* 202
ii. Average macroconidia 45.6 μ long...................*F. angustum* 203
hh. Conidiophores typically much branched
i. In plate cultures on neutral potato agar producing exposed and distinct pseudopionnotes
j. Color of the pseudopionnotes vinaceous red.*F. redolens* var. *Solani* 205
jj. Color of the pseudopionnotes vinaceous purple......*F. lutulatum* 209
ii. No exposed and distinct pseudopionnotes in potato agar plate cultures
j. Macroconidia typically somewhat broader toward apex........ *F. sclerotioides* 214
jj. Macroconidia typically not broader toward apex...*F. oxysporum* 220
ee. Conidia with more or less abruptly attenuated apex, rounded or papillate
f. Substratum typically (on glucose agar) carmine red. The color may be yellowish, but never gray, green, nor blue............Section **Discolor** 228
g. Conidia non- to three-septate, rounded at both ends, of common type, numerous (those of discolor type usually only few)..*F. trichothecioides* 229
gg. Discolor type of conidia common, and nearly the only type present
h. Conidial masses pale cream to pale pink in color, aërial mycelium well developed and nearly white.......................*F. subpallidum* 230

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hh. Conidial masses of pale orange to dark chocolate-red in color, mycelium from nearly slightly pinkish to dense carmine red
  i. Conidia much broadened toward apex . . . . *F. clavatum* 234
  ii. Conidia not or only slightly broadened toward apex
    j. Average diameter of five-septate conidia from 4 to 4.4 μ . . . . *F. discolor* 236
    jj. Average diameter of five-septate conidia from 5.8 to 6.8 μ . . . . . . *F. culmorum* 240
ff. Substratum typically (on a neutral or not strongly acid glucose agar) brownish gray, vinaceous red, vinaceous purple, or blue, but never carmine red . . . . Section Martiella 244
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gg. Macroconidia typically somewhat broader toward basal end . . . . *F. coeruleum* 260

DESCRIPTIONS OF SECTIONS, GENERA, SPECIES, AND VARIETIES

FUSARIUM Link

Link, Mag. Ges. nat. Freunde **3**:10. 1824. Saccardo, Syll. Fung. **4**:694. 1886. (Cf. Appel. O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:60–61. 1910. Wollenweber, H. W., Phytopath. **3**:24–50, 197–240, fig. 1, Pls. I and III. 1913; Ber. deut. Bot. Gesell. **31**:17–34. 1913. Journ. Agr. Research **2**:251–285. 1914.)

Hyphomycetes, with from hyaline to bright, but never plain gray nor black, conidia and mycelium; conidia sickle-shaped, septate (usually 3– or more septate), apically pointed, mostly pedicellate, not appendiculate, noncatenulate; conidia scattered over substratum, in pseudopionnotes or in sporodochia, the latter without or with from flat to wartlike plectenchymic substratum, and always without any differentiated enclosing or surrounding structures; conidiophores from simple to irregularly verticillate.

I. SECTION **DIMERUM** n. sec.

Conidia dorsiventral, 1–septate; chlamydospores may be present.

This section is proposed in order that Fusaria with 1–septate conidia may be included. The two species of this section are comparatively slow-growing fungi, with hyaline or nearly hyaline mycelium and from hyaline to orange-colored conidia. They appear to differ in many ways from typical Fusaria, but the differences are not sharp enough to warrant transferring them into another genus.

1. *Fusarium affine* Faut. et Lamb. (Figs. 1D and 2)

Fautrey, F., and Lambotte, E., Espèces nouvelles ou rares de la Côte-d'Or, Rev. Myc. Fr. 18:68. 1896. Saccardo, Syll. Fung. 14:1125. 1899.

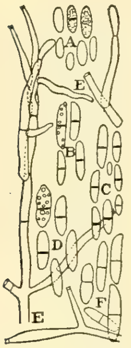

FIG. 2.—*Fusarium affine.* A, *Conidia from 35-days-old culture on slightly acidified potato agar;* B, *conidia from 7-days-old plate culture on hard potato agar;* C, *conidia from 74-days-old culture on red raspberry cane plug;* D, *conidia from 4-days-old culture on hard lima-bean agar;* E, *conidiophores from various media;* F, *conidia from 5-days-old culture on nutrient agar*

Conidia straight, somewhat dorsiventral near apex, apedicellate, typically 1–septate, 10.2 x 2.8 (9–11.4 x 2.6–3) μ, usually in a continuous smooth or slightly roughened, slimy layer, from hyaline to pale salmon-colored on a glucose agar; conidiophores from simple to sparingly branched, septate; mycelium hyaline; no chlamydospores.

Hab. In tubers and stems of *Solanum tuberosum,* in greenhouse soil, New York.

This organism was repeatedly isolated from various sources such as discolored fibrovascular bundles of potato tubers, from the base of wilted potato stems, and from soil. Its size is exactly the same as that given by Wollenweber (1913 c:229) for the conidial stage of *Mycosphaerella Solani* (E. et E.) Wr. Wollenweber's illustrations (Plate XXI, fig. N) are also much the same, and he considers *F. affine* Faut. et Lamb. as the conidial stage. The strain studied did not show any perfect form in culture, although it was grown for more than a year and on various media. This organism can be at once distinguished from all the other Fusaria by its minute, 1–septate, nearly straight conidia and its inconspicuous, slow growth.

For a fuller presentation of its septation and size the following detailed measurements are given:

On red raspberry cane plug, culture seventy-four days old:

Conidia: 0–septate, 45 per cent, 7 x 2.2 (3.5–9 x 1.7–2.6) μ
1–septate, 55 per cent, 9 x 2.6 (6–12 x 2–3) μ

On hard lima-bean agar, culture four days old:
Conidia: 0–septate, 40 per cent
1–septate, 60 per cent, 10.2 x 3 (9–11 x 2.8–3.8)μ

On nutrient agar, culture six days old:
Conidia: 0–septate, 10 per cent
1–septate, 90 per cent, 11.4 x 2.8 (8.7–13 x 2.4–3.5)μ

Average of the above measurements:
Conidia: 0–septate, 32 per cent, 7 x 2.2μ
1–septate, 68 per cent, 10.2 x 2.8μ

The size of the spores of *F. affine* as given by Saccardo (10–15 x 4μ) differs somewhat from the above, but the description otherwise is so much the same that the organisms can be considered identical.

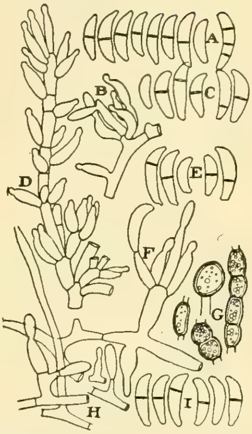

FIG. 3.—*Fusarium dimerum.* A, *Pseudopionnotal conidia from 70-days-old culture;* B, *conidiophore,* C, *pseudopionnotal conidia, from 19-days-old culture on slightly acidified hard potato agar;* D, *conidiophore from 70-days-old culture on potato stem plug;* E, *conidia,* F, *conidiophore, from 43-days-old culture on hard lima-bean agar with 2 per cent glucose;* G, *chlamydospores;* H, *conidiophores,* I, *conidia, from 21-days-old culture on hard lima-bean agar*

2. *Fusarium dimerum* Penz. (Figs. 1E and 3)

Penzig, O., Michelia 2:484. 1882. Saccardo, Syll. Fung. 4:704. 1886. Lindau, Rab. Krypt. Fl. Pilze 9:566. 1910. Appel and Wollenweber, Arb. K. biol. Anst. Land- u. Forstw. 8:37, text figs. 2 and 4. 1910.

Conidia lunar, somewhat pedicellate, typically 1–septate, 13 x 3.3 (12.5–13.5 x 3.3–3.4)μ, often also 0–septate, rarely 2– or 3–septate, borne singly on the mycelium or forming a more or less continuous slimy layer, from hyaline to cinnamon-buff on glucose agar; mycelium from hyaline to about the color of the conidial masses; chlamydospores intercalary, in mycelium.

Hab. On tubers and stems of *Solanum tuberosum* in Germany and in Minnesota (U. S. A.), and on fruits of *Citrus medica* in Italy.

The fungus is easily distinguished from all the other Fusaria by its lunar, minute, 1–septate conidia. It was isolated by the author only once, from a superficial dry rot of potato tuber received from St. Paul, Minnesota.

The measurements of the conidia in detail are as follows:

On hard potato agar, slightly acidified, culture eighteen days old:
Conidia: 0–septate, 3 per cent
1–septate, 97 per cent, 12.5 x 3.4 (10–18 x 2.9–4.1)μ

On hard lima-bean agar with 2 per cent glucose, culture forty-three days old:
Conidia: 0–septate, 12 per cent, 10 x 3.8μ
1–septate, 88 per cent, 12 x 3.3 (10–18 x 3–3.5)μ

On hard lima-bean agar, culture twenty-one days old:
Conidia: 0–septate, 27 per cent, 11.5 x 3.2 (9–14 x 2.9–3.5)μ
1–septate, 73 per cent, 12.5 x 3.3 (10–17 x 2.9–3.9)μ

Average of the above measurements:
Conidia: 0–septate, 14 per cent, 10.8 x 3.5μ
1–septate, 86 per cent, 12.8 x 3.3μ

The organism studied is so much the same as *F. dimerum* originally described by Penzig, that it is considered identical. A similar organism is also reported as occurring on potato in Germany (Appel and Wollenweber, 1910:37). The only peculiarity of the organism which either was not observed or was not present at all in the original *F. dimerum* is that the septum in the conidia of the organism studied is often and conspicuously acentric.

II. Section Ventricosum Wr., Phytopath. 3:32

Conidia only slightly dorsiventral, somewhat wedge-shaped, broader toward base, apex somewhat rounded, apedicellate, typically 3–septate; no sporodochia; chlamydospores variable,[38] always present.

3. *Fusarium ventricosum* Ap. et Wr.

Wollenweber, H. W., Phytopath. 3:32, fig. 1, A and V. 1913.

Wollenweber describes this species as follows: "Conidia never formed in sporodochia, brownish-white to cream-colored, 3–septate, 29–37 x 5.75–7.5μ; conidiophores bostryx-like or irregularly branched, chlamydospores like those of the section Elegans. Wound parasite, ... found in Europe. Inhabits also *Beta vulgaris*."

The organism was neither isolated nor studied by the writer.

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[38] According to Wollenweber the chlamydospores are of Elegans type, that is, terminal and intercalary, 0– to 1– or more septate. In order to include *F. cuneiforme* it was necessary to alter the characterization of the chlamydospores in this section.

4. Fusarium cuneiforme n. sp. (Figs. 1 F and 4)

Conidia only slightly dorsiventral, more or less wedge-shaped, broader toward the base, with apex somewhat rounded, apedicellate, typically 3-septate, 34.7 x 5.6 (30–41 x 5.6–6)μ, often 0- to 2-septate, in false balls, from hyaline to cream-colored; no sporodochia; aërial mycelium hyaline, in a high tuft in center, and short, distinctly zonate, outside; chlamydospores from smooth to very distinctly warted, sometimes surrounded with a gelatinous capsule, terminal only, typically unicellular, 8.2 x 7.6 (7.6–8.5 x 7.3–8.1)μ.

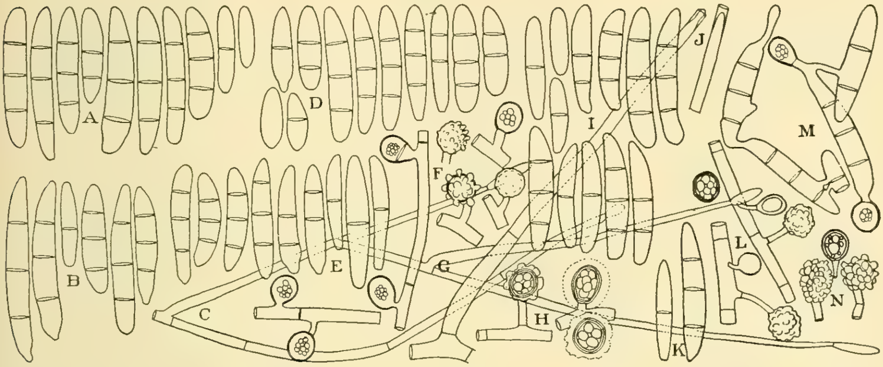

Fig. 4.— *Fusarium cuneiforme.* A, *Conidia from false balls of 16-days-old culture on slightly acidified hard potato agar;* B, *conidia from false balls from 10-days-old potato agar with 0.5 per cent glucose;* C, *conidiophores,* D, *conidia, from a thin layer of 73-days-old culture on red raspberry cane plug;* E, *conidia from confluent thin mass of false balls of 64-days-old culture on hard bean agar;* F, *terminal chlamydospores,* G, *conidiophore, from 34-days-old culture on hard lima-bean agar;* H, *terminal chlamydospores of 16-days-old culture on slightly acidified hard potato agar;* I, *conidia,* J, *tips of conidiophores showing beginning of production of new conidia, from 34-days-old culture on hard lima-bean agar;* K, *conidia,* L, *terminal chlamydospores, from 26-days-old corn agar;* M, *anastomosis, and chlamydospore producing conidia from 34-days-old culture on hard lima-bean agar;* N, *terminal chlamydospores from the same culture*

Hab. The fungus was isolated from soft rotted potato tubers received from Auburn, Alabama, and from Atlanta, New York, always in association with bacteria and other fungi.

Differs from *F. ventricosum* Ap. et Wr. mainly in typically 0-septate chlamydospores which are terminal only. There seems to be some difference also in the shape and size of the conidia, which here are somewhat more slender.

Latin description.—Conidiis parum dorsiventralibus, plus minusve cuneiformibus, deorsum latioribus, apice subrotundato, apedicellatis, typice 3–septatis, 34.7 x 5.6 (30–41 x 5.6–6)μ, 0–2–septatis, globis falsis, ex hyalino "cream color" (R); nullis sporodochiis; aerio mycelio hyalino, medio alte cristato, extra brevi et distincte zonato; chlamydosporis levibus vel maxime distincte verrucosis, interdum capsula gelatinosa cinctis, tantum terminalibus, typice unicellularibus, 8.2 x 7.6 (7.6–8.5 x 7.3–8.1)μ.

Hab. Fungus ex tuberibus mollibus putridisque Solani tuberosi ab Auburn, Alabama, et Atlanta, New York, Amer. bor. receptis, semper una cum bacteriis aliisque fungis, sejungebatur.

The measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture sixteen days old:
Conidia: 0–septate, rare, 16 x 4μ
1–septate, rare, 21 x 5.2μ
2–septate, 1 per cent, 24 x 5.6 (20–32 x 5.2–5.8)μ
3–septate, 99 per cent, 35.5 x 6 (24–44 x 4.7–7.5)μ

On red raspberry cane plug, culture seventy-three days old:
Conidia: 0–septate, 12 per cent, 14 x 5.9 (6.5–17 x 5–6.5)μ
1–septate, 34 per cent, 19 x 6 (15–27 x 4.8–7)μ
2–septate, 11 per cent, 33 x 5.9 (20–40 x 5.2–6.5)μ
3–septate, 43 per cent, 33 x 5.9 (20–40 x 5.2–6.5)μ

On hard potato agar, culture twenty-five days old:
Conidia: 1–septate, rare
3–septate, 100 per cent, 35 x 5.8 (24–48 x 5–6.5)μ

On hard lima-bean agar with 2 per cent glucose, culture sixty-four days old:
Conidia: 0–septate, 4 per cent
1–septate, 64 per cent, 25 x 4.9 (12–41 x 3.5–6.5)μ
2–septate, 22 per cent, 28 x 5.5 (23–32 x 4.8–5.9)μ
3–septate, 10 per cent, 30 x 5.6 (24–38 x 4.8–6.2)μ

On hard lima-bean agar, culture thirty-four days old, advanced part of colony growth:
Conidia: 0–septate, rare
1–septate, 17 per cent
2–septate, 7 per cent
3–septate, 76 per cent, 31.5 x 6 (27–41 x 5.2–7.6)μ

On same culture as above but from an old part of the colony growth:
3–septate, 70 per cent, 41 x 6 (36–46 x 5.2–7.6)μ

Average of the above measurements:
Conidia: 0–septate, 3 per cent, 15 x 5μ
1–septate, 23 per cent, 21.3 x 5.4μ
2–septate, 8 per cent, 26 x 5.45μ
3–septate, 66 per cent, 34.7 x 5.9μ

III. SECTION EUPIONNOTES Wr., Phytopath. 3:38, 206, 219

Conidia dorsiventral, apedicellate, nearly cylindrical for the largest part or slightly broader toward apex, typically 3–septate, in true pionnotes; terminal and intercalary chlamydospores present. Differs from all the other sections of Fusaria by true pionnotal fruiting form.

5. *Fusarium udum* (Berk.) Wr.

Syn. *Fusisporium udum* Berk., Ann. Mag. Nat. Hist. 6:438, Pl. XIV, fig. 28. 1841.
Pionnotes uda (Berk.) Sacc., Syll. Fung. 4:726. 1886.

Cf. Wollenweber, Phytopath. 3:38 (footnote), 219 (key), Pl. XXI, figs. R and S.

According to Wollenweber's data and figures, the conidia are dorsiventral with somewhat rounded apex, apedicellate, typically 3–septate, about 33–45 x 3.5–4μ, sometimes 4– or 5–septate; terminal chlamydospores present.

Hab. On cut surfaces of oak, elm, and other trees, also on *Solanum tuberosum*, on tulip bulbs, and in the soil.

6. *Fusarium udum* (Berk.) Wr. var. **Solani** n. var. (Figs. 1G and 5)

Conidia dorsiventral, usually somewhat broader toward the slightly rounded apex, apedicellate, typically 3–septate, 30.2 x 4.27 (25–34 x 4–4.5)μ, non– to two–septate very rare when mature, 4– and 5–septate rare, of from light vinaceous cinnamon to orange-cinnamon hues on agars rich in glucose; chlamydospores usually found only in old cultures, terminal and intercalary, in conidia, in the tips of sterigmata, and in mycelium, often of dense orange color, 0–septate, 6 x 5.5μ; aërial mycelium present only near margin of colony growth, very loose, short, hyaline; substratum colorless or approaching the color of the conidia.

Hab. On rotted tubers of *Solanum tuberosum*, together with *Ramularia Solani*, Long Island, New York.

Differs from *F. udum* (Berk.) Wr. in that the conidia are shorter and thicker, and somewhat broader toward the apex.

The following measurements were taken:

On slightly acidified hard potato agar, culture eight days old:

Conidia: 0– and 1–septate, only when young
3–septate, 100 per cent, 33 x 4.25 (28–39 x 3.5–4.7)μ

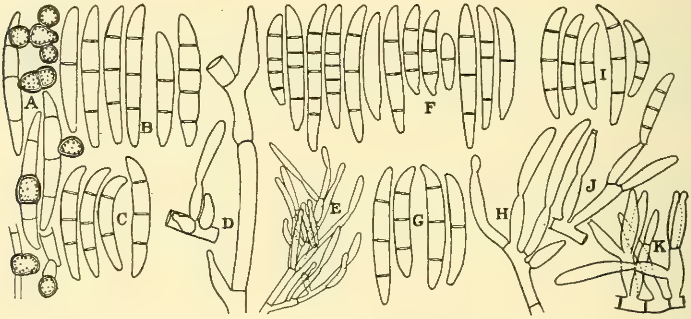

Fig. 5.—*Fusarium udum var. Solani.* A, *Different forms of chlamydospores from 85-days-old culture on hard potato agar with 5 per cent glucose;* B, *conidia from 9-days-old culture on hard lima-bean agar;* C, *conidia,* D, *conidiophores, from 49-days-old culture on rye straw;* E, *conidiophores (magnified 250 times) from 8-days-old culture on slightly acidified hard potato agar;* F, *conidia from 72-days-old culture on potato tuber plug;* G, *conidia,* H, *conidiophores, from 8-days-old culture on slightly acidified hard potato agar;* I, *conidia,* J, K, *conidiophores, from 74-days-old culture on red raspberry cane plug*

On red raspberry cane plug, culture seventy-four days old:

(1) From an advanced part of colony growth

Conidia: 1–septate, rare
2–septate, 10 per cent, 22 x 3.8 (19.5–24 x 3.3–4.1)μ
3–septate, 90 per cent, 25 x 4 (22–31 x 3.9–4.6)μ

(2) From an old part of colony growth

Conidia: 3–septate, 100 per cent, 28 x 4μ dominant type
4–septate, rare

On potato tuber plug, culture seventy-two days old:
Conidia: 0–septate, rare, young
1–septate, 5 per cent, mostly young
2–septate, 2 per cent, mostly young
3–septate, 86 per cent, 31 x 4.4 (23–40 x 3.9–4.7)μ
4–septate, 7 per cent, 32 x 4.4 (28–40 x 4.1–4.7)μ
5–septate, rare, 35 x 4.4 (30–40 x 4–4.6)μ

On hard lima-bean agar, culture four days old:
Conidia: 1–septate, 1 per cent, young
2–septate, 4 per cent, 31 x 4.4
3–septate, 95 per cent, 34 x 4.5 (25–40 x 4.1–4.9)μ
4–septate, rare, 40 x 5.1 (only a few measured)

Average of the above measurements:
Conidia: 0–septate, rare to none
1–septate, 1.5 per cent, (usually immature)
2–septate, 4 per cent
3–septate, 93 per cent, 30.2 x 4.27μ
4–septate, 1.5 per cent, 36 x 4.75μ
5–septate, rare to none

IV. SECTION GIBBOSUM Wr., Phytopath. **3**:31, fig. 1, L and M

Conidia with from hyperbolic or parabolic to elliptic dorsal curve, conspicuously broader in the middle, with more or less long, narrow apex, prominently pedicellate, mostly 5–septate; intercalary chlamydospores always present; color of substratum and conidial mass typically from pale buff to cinnamon and sepia; mycelium from hyaline to brown.

7. *Fusarium gibbosum* Ap. et Wr. (Figs. 1Y and 6)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:185–190, text fig. 10, C and D. 1910. Wollenweber, H. W., Phytopath. **3**:32, fig. 1, M. 1913.

Conidia often with hyperbolic dorsal curve, conspicuously broader in the middle, with long, narrow apex, prominently pedicellate, typically 5-septate, 41.6 x 4.6 (40–46 x 4.4–4.7)μ, sometimes also 0– to 7–septate, in minute nonconverging sporodochia or spreading over mycelium singly, from hyaline to light pinkish cinnamon in color; chlamydospores intercalary, always present; aërial mycelium typically present, short, fine, medium loose; colony faintly zonate; substratum on potato agar rich in glucose, from pale flesh to cinnamon in color.

Hab. On stems and tubers of *Solanum tuberosum* in Germany, rare.

The organism was not isolated by the writer, but a culture of it was carefully examined on several different media with the following results in regard to spore septation and size:

On slightly acidified hard potato agar, culture twenty-four days old:

Conidia: 3–septate, 5 per cent
4–septate, 15 per cent, 32–4.1μ
5–septate, 80 per cent, 43.2 x 4.6 (38–50 x 4.3–4.9)μ

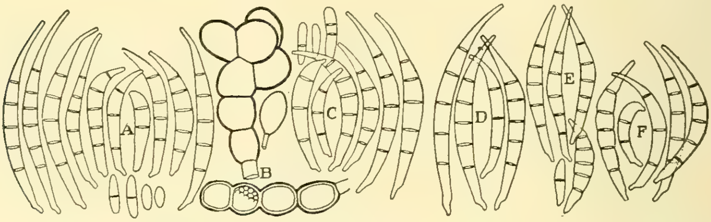

FIG. 6.—*Fusarium gibbosum.* A, *Pseudopionnotal conidia from 10-days-old culture on hard lima-bean agar;* B, *intercalary chlamydospores,* C, *conidia, from 71-days-old culture on red raspberry cane plug;* D, *pseudopionnotal conidia from 118-days-old culture on hard lima-bean agar with 2 per cent glucose;* E, *conidia from 42-days-old culture on potato stem plug;* F, *pseudopionnotal conidia from 24-days-old culture on slightly acidified hard potato agar*

On red raspberry cane plug, culture seventy-one days old:

Conidia: 3–septate, 5 per cent
4–septate, 5 per cent
5–septate, 90 per cent, 40 x 4.6 (33–46 x 4.2–5.2)μ
6–septate, rare

On hard lima-bean agar with 2 per cent glucose, culture one hundred and eighteen days old:

Conidia: 4–septate, 1 per cent
5–septate, 94 per cent, 37 x 4.6 (26–44 x 3.9–4.9)μ
6–septate, 3 per cent, 42 x 4.7μ
7–septate, 2 per cent

On hard lima-bean agar, culture ten days old:

Conidia: 0–septate, 1.5 per cent, 7.5 x 3.2 μ
1–septate, 4 per cent, 10 x 3.2 (7–17 x 2.7–3.5)μ
2–septate, very rare
3–septate, 4 per cent, 27 x 4.1 (19–41 x 3.5–4.1)μ
4–septate, 3 per cent
5–septate, 86 per cent, 46 x 4.4 (35–58 x 4–4.8)μ
6–septate, 1.5 per cent, 52 x 4.5 (50–60 x 4.3–4.8)μ

Average of the above measurements:

Conidia: 0–septate, 0.5 per cent, 7.5 x 3.2μ
1–septate, 1 per cent, 10 x 3.2μ
2–septate, very rare
3–septate, 4 per cent, 27 x 4.1μ
4–septate, 6 per cent, 27 x 4.1μ
5–septate, 87 per cent, 41.6 x 4.6μ
6– and 7–septate, 1.5 per cent, 47 x 4.6μ

The averages of Appel and Wollenweber's (1910:189–190) measurements are as follows:

Conidia: 3–septate, 3 per cent, 25 x 4.5μ
4–septate, 2 per cent, 29 x 4.5μ
5–septate, 59 per cent, 42 x 4.4μ
6–septate, 23 per cent, 49 x 4.6μ
7–septate, 13 per cent, 49 x 4.7μ

There is a notable difference between the data presented by the writer and those of Wollenweber only in the proportion of 6– and 7–septate conidia, the size of 5– and of 6– and 7–septate conidia being very much the same.

8. *Fusarium falcatum* Ap. et Wr. (Figs. 1x and 7)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. 8:175–185; Pl. II, figs. 100 to 110; Pl. III, fig. 9; text fig. 10, A. 1910.

Syn. *Fusarium vasinfectum* var. *Pisi* Schikorra, Arb. K. biol. Anst. Land- u. Forstw. 4:157, Pl. VII, 1906; not *F. vasinfectum* var. *Pisi* van Hall, Ber. deut. Bot. Gesell. 21: 4, pl. 4, 1903. Wollenweber, Phytopath. 3:31, fig. 1, L, 1913.

Conidia often with parabolic dorsal curve, conspicuously broader in the middle, with long and narrow apex, prominently pedicellate, typically 5-septate, 49.1 x 4.6 (43–54.5 x 4.5–4.7)μ, often 3- to 7-septate, 0- to 2- and 8-septate very rare, in minute sporodochia more or less converging into pseudopionnotes, from buff-pink to cinnamon on hard potato agar rich in glucose; intercalary chlamydospores always present; aërial mycelium very poorly developed or not developed at all, leaving slimy layer exposed; substratum about the same color as the spores.

Hab. Often on *Pisum sativum*, seldom on underground part of stem of *Solanum tuberosum*, in Germany, and cause of fruit rot of *Solanum lycopersicum* in Germany and in the United States.

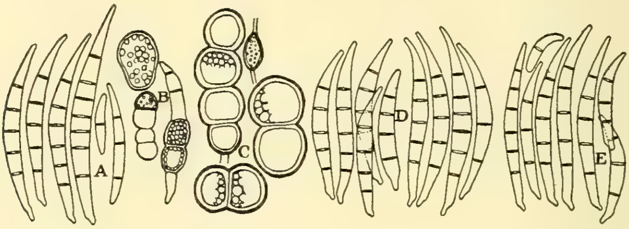

FIG. 7.—*Fusarium falcatum.* A, *Pseudopionnotal conidia,* B, *chlamydospores in mycelium and in spore, from 119-days-old culture on hard lima-bean agar with 2 per cent glucose;* C, *chlamydospores,* D, *pseudopionnotal conidia, from 71-days-old culture on red raspberry cane plug;* E, *pseudopionnotal conidia from 42-days-old culture on potato stem plug*

Differs from *F. gibbosum* mainly in having parabolic dorsal curve and typically exposed pseudopionnotes; also in considerably longer conidia.

The organism was not isolated by the writer, but the culture was obtained through the courtesy of Dr. Wollenweber. The writer's cultural observations in regard to septation and size of spores of this organism on various media are as follows:

On slightly acidified hard potato agar, culture twenty-four days old:

Conidia: 3-septate, 3 per cent, 28–42 x 3.5–4μ } (only a few measured)
4-septate, 9 per cent, 33–42 x 3.5–4μ }
5-septate, 77 per cent, 50 x 4.5 (43–58 x 4–5.3)μ
6-septate, 10 per cent, 54 x 5 (50–60 x 4.8–5.3)μ
7-septate, 1 per cent, about 60–70 x 4.8–5.3μ

On red raspberry cane plug, culture seventy-one days old:

Conidia: 3–septate, 2 per cent
4–septate, 3 per cent
5–septate, 90 per cent, 43 x 4.6 (36–49 x 4–5.2)μ
6–septate, 5 per cent, 46 x 4.8 (43–53 x 4.3–5.2)μ

On hard lima-bean agar with 2 per cent glucose, culture one hundred and nineteen days old:

Conidia: 1–septate, 2.5 per cent, 15 x 2.7μ (only two measured)
3–septate, 3 per cent, 30 x 3.6 (10–44 x 3–4.7)μ
4–septate, 5 per cent
5–septate, 81 per cent, 49 x 4.7 (42–55 x 4.3–5.2)μ
6–septate, 6 per cent, 51 x 4.9 (45–56 x 4.6–5.2)μ
7–septate, 2.5 per cent, 52 x 5.2 (47–58 x 5.2)μ
8–septate, exceptionally rare, 58 x 5.4 (only one measured)

On hard lima-bean agar, culture eleven days old:

Conidia: 0–septate, rare
2–septate, 2 per cent
3–septate, 30 per cent, 30 x 3.6 (20–40 x 3–4.3)μ
4–septate, 5 per cent
5–septate, 62 per cent, 54.5 x 4.5 (43–65 x 3.5–5.2)μ
6–septate, 1 per cent, 64 x 4.8 (59–70 x 4.6–5.3)μ
7–septate, rare, same as 6–septate

Average of the above measurements:

Conidia: 0–septate, very rare
1–septate, 0.5 per cent
2–septate, 0.5 per cent, 15 x 2.7μ
3–septate, 9.5 per cent, 30 x 3.6μ
4–septate, 5.5 per cent
5–septate, 77.5 per cent, 49.1 x 4.56μ
6–septate, 5.5 per cent, 54 x 4.9μ
7–septate, 1 per cent, 58 x 5.1μ
8–septate, very rare, 58 x 5.4μ

Averages of Appel and Wollenweber's (1910:184) measurements are as follows:

Conidia: 5–septate, 46 x 4.7μ
6–septate, 49 x 4.4μ

The measurements of the writer show some deviation from these, but the deviation is small and can be explained entirely by the fact that the writer's measurements were taken from much younger cultures, which usually yield somewhat longer conidia than those produced in old cultures.

9. *Fusarium falcatum* Ap. et Wr. var. **fuscum** n. var. (Fig. 8; Pl. VII, fig. 8)

Conidia with from ellipsoidal to parabolic dorsal curve, conspicuously broader in the middle, prominently pedicellate, typically 5–septate, 45 x 4.6 (40–51 x 4.4–4.7)μ, 3– and 4–septate ones also present, 6– and 7–septate rare, 8–septate very rare, typically in conspicuous plectenchymic or aplectenchymic sporodochia, from light buff and honey yellow to buckthorn and cacao brown, on potato agar rich in glucose; chlamydospores intercalary, always present, sometimes by their abundance and color making the entire medium and the aërial mycelium of a dark brown color; aërial mycelium always present, high, from fine to medium fine, more or less loose, hyaline at first, later becoming from tawny olive to brown; color of substratum on agars from hyaline to that of the spores.

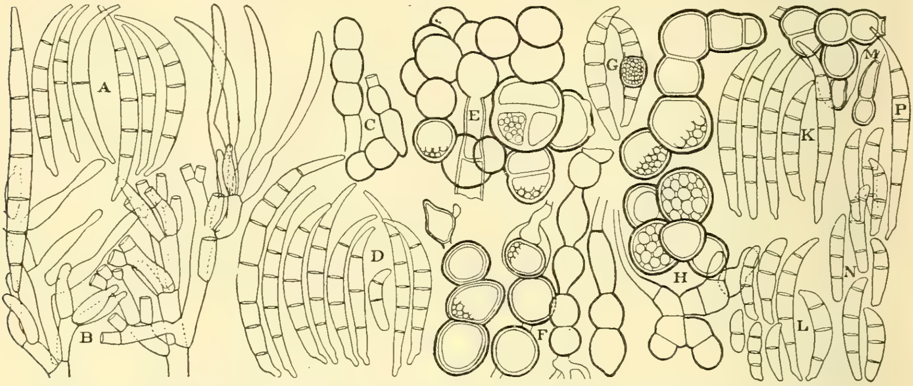

FIG. 8.—*Fusarium falcatum var. fuscum.* A, *Pseudopionnotal conidia,* B, *conidiophores, from 4-days-old culture on hard potato agar;* C, *intercalary chlamydospores,* D, *pseudopionnotal conidia, from 10-days-old culture on hard bean agar;* E, *cluster of intercalary chlamydospores from 99-days-old culture on potato tuber plug;* F, *intercalary chlamydospores in long chains,* G, *conidia with and without chlamydospores, from 176-days-old culture on corn agar;* H, *young and old chlamydospores,* K, *sporodochial,* L, *aërial, conidia, from 79-days-old culture on red raspberry cane plug;* M, *intercalary and terminal chlamydospores,* N, *aërial conidia, from 50-days-old culture on rye straw;* P, *typical conidium from a large sporodochium of 29-days-old culture on hard oat agar*

Hab. On rotted tubers of *Solanum tuberosum*, New York State.

Differs from *F. falcatum* mainly by large sporodochia, more profuse chlamydospore production, well-developed aërial mycelium, and typical absence of pseudopionnotes.

The organism was isolated only once, from a potato tuber slightly rotted near the stem end, which was received from a potato grower in New York. The measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture four days old:

Conidia: 0– and 1–septate, few, very young
3–septate, 4 per cent, 38 x 4.3 (35–42 x 4.1–4.7)μ
4–septate, 6 per cent, 41.6 x 4.4 (35–44 x 4.1–4.7)μ
5–septate, 88 per cent, 45.5 x 4.6 (40–49 x 4.1–4.8)μ
6– and 7–septate, 2 per cent, 50–80 x 5.5μ (only a few measured)
The largest 5–septate conidium observed measured 67 x 5.8μ

On red raspberry cane plug, culture seventy-nine days old:

Conidia: 3–septate, 1 per cent
4–septate, 4 per cent
5–septate, 95 per cent, 40 x 4.7 (31–48 x 4.3–5.2)μ

On hard lima-bean agar, culture ten days old:

Conidia: 3–septate, 3 per cent, 34 x 4.4 (19–40 x 3.1–4.7)μ
4–septate, 6 per cent
5–septate, 91 per cent, 45 x 4.4 (36–61 x 4–4.8)μ

On hard oat agar, culture twenty-nine days old, from a sporodochium about 3 millimeters in diameter:

Conidia: 3–septate, 2 per cent
4–septate, 5 per cent
5–septate, 93 per cent, 51 x 4.6 (45–53 x 4.1–4.9)μ

Average of the above measurements:

Conidia: 3–septate, 2.5 per cent, 36 x 4.3μ
4–septate, 5 per cent, 41.6 x 4.4μ
5–septate, 92 per cent, 45 x 4.6μ
6– and 7–septate, 0.5 per cent

10. *Fusarium caudatum* Wr. var. **Solani** n. var. (Figs. 1z and 9; Pl. IV, fig. 7; Pl. VI, fig. 3)

Conidia with from parabolic to ellipsoid dorsal curve, conspicuously broader at the middle, with very long, narrow, whiplike apex, prominently pedicellate, typically 5– to 7–septate; measuring on the average, 5–septate, 48 x 4.6 (40–55 x 4.3–4.7)μ, 7–septate, 64.7 x 4.6 (57–69 x 4.4–4.8)μ; rarely in pseudopionnotes, typically in small aplectenchymic sporodochia, tinted from cream-buff to cinnamon, clay, and Saccardo's amber in a plate culture on a potato hard agar rich in glucose; chlamydospores intercalary, always present in greater or less abundance; aërial mycelium very well developed, high, uniform, medium dense, from hyaline when young to sepia in old cultures, mostly from brownish to dresden brown; substratum, on potato agar rich in glucose, from pinkish buff when young to ochraceous tawny and snuff brown shaded to sepia in very old cultures.

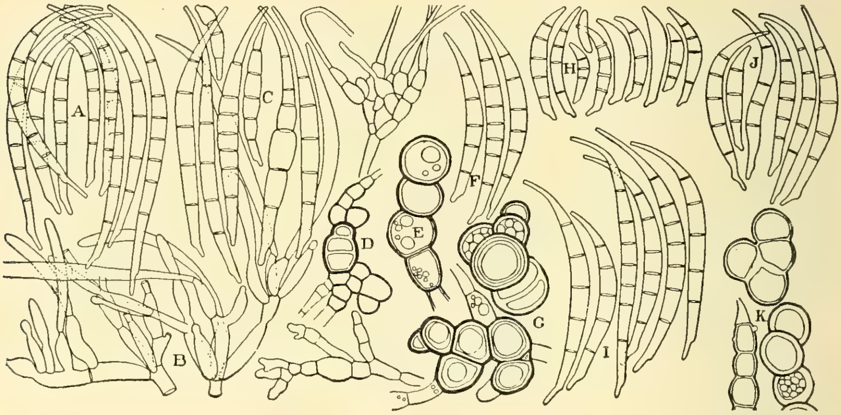

FIG. 9.—*Fusarium caudatum var. Solani.* A, *Pseudopionnotal conidia,* B, *conidiophores, from 9-days-old culture on slightly acidified hard potato agar;* C, *pseudopionnotal conidia from 38-days-old culture on potato stem plug;* D *and* E, *chlamydospores from 50-days-old culture on red raspberry cane plug (magnification 250 times);* F, *conidia from aërial sporodochium of 118-days-old culture on hard lima-bean agar with 2 per cent glucose;* G, *cluster of chlamydospores,* H, *aërial conidia, from 50-days-old culture on rye straw;* I, *sporodochial conidia in oozing drop of liquid from 9-days-old culture on hard lima-bean agar;* J, *aërial conidia,* K, *intercalary chlamydospores, from 69-days-old culture on red raspberry cane plug*

Hab. On superficial dry-rot spots on tubers of *Solanum tuberosum,* Atlanta, New York.

Differs from *F. caudatum* Wr. (see Wollenweber 1914:262–263, Pl. XVI, fig. M) mainly by broader conidia, which in the latter organism average only from 3 to 4.5μ in diameter.

The organism was twice isolated, in 1912 and in 1913, from superficial dry rot of potato tubers collected at Atlanta, New York. The measurements of conidia on different media are as follows:

On red raspberry cane plug, culture sixty-nine days old:
Conidia: 3–septate, 10 per cent, 30 x 4 (26–35 x 3.5–4.5)μ
4–septate, 5 per cent, 34 x 4.4 (32–40 x 4–4.8)μ
5–septate, 85 per cent, 40 x 4.8 (33–55 x 4–5.4)μ

On slightly acidified hard potato agar, culture nine days old:
Conidia: 3–septate, 1 per cent, about 38 x 4μ (only a few measured)
4–septate, 8 per cent, about 40 x 4.2μ (only a few measured)
5–septate, 43 per cent, 55 x 4.3 (38–69 x 3.5–5.9)μ
6–septate, 27 per cent, 65 x 4.4 (55–84 x 4.1–5.3)μ
7–septate, 21 per cent, 69 x 4.4 (60–85 x 4.1–5.9)μ
8–septate, very rare

On hard lima-bean agar with 2 per cent glucose, culture one hundred and eighteen days old:
Conidia: 4–septate, 1 per cent
5–septate, 29 per cent, 46 x 4.7 (43–59 x 4.3–5.2)μ
6–septate, 32 per cent, 53 x 4.8 (48–60 x 4.3–5)μ
7–septate, 37 per cent, 57 x 4.8 (50–62 x 4.7–5)μ
8–septate, 1 per cent, 61 x 5μ (only one measured)

On hard lima-bean agar, culture nine days old:
Conidia: 5–septate, 55 per cent, 52 x 4.5 (45–61 x 4–4.8)μ
6–septate, 35 per cent, 65 x 4.7 (55–71 x 4.3–4.8)μ
7–septate, 10 per cent, 68 x 4.7 (58–78 x 4.3–4.8)μ

Average of the above measurements:
Conidia: 3–septate, 3 per cent, 34 x 4μ
4–septate, 3.5 per cent, 37 x 4μ
5–septate, 53 per cent, 48 x 4.6μ
6–septate, 23.5 per cent, 58 x 4.6μ
7–septate, 17 per cent, 64.7 x 4.6μ
8–septate, rare, 61 x 5μ (only one measured)

V. SECTION ROSEUM Wr. (emended), Phytopath. 3:32, fig. 1 N, 1913

Conidia broad ellipsoid, typically of an even diameter for a considerable part of their length, comparatively narrow (from 3.6 to 4.3μ in average diameter), always very gradually attenuate toward both ends, conidia of all fruiting forms of the same shape and type; true chlamydospores always absent; on agars rich in glucose, from honey yellow and morocco red to Eugenia red, sometimes nearly hyaline.

11. *Fusarium acuminatum* Ell. et Ev. emend. Wr.

Cf. Wollenweber, H. W., Journ. Agr. Research 2:269–270, Pl. XVI, fig. G. 1914. *Fusarium acuminatum* Ell. et Ev., Proc. Acad. Sci. Phila. 1895:441. Saccardo, Syll. Fung. 14:1125–1126. 1899. Wollenweber's diagnosis (page 269 of reference cited) is as follows:

" Conidia, scattered, in sporodochia or in pionnotes, orange in mass. Conidia average as follows: 5–septate, 40 to 70 by 3 to 4.5μ; 4–septate (less common), 30 to 60 by 3 to 4.5μ; 3–septate, 20 to 45 by 2.75 to 4.25μ. Conidia of 0–, 1–, 2–, 6–, and 7–septations are occasionally found. Subnormal small conidia may be mistaken for conidia of the section Discolor, but normal sporodochia develop on repeatedly whorl-like branched conidiophores, giving the characteristic conidia of the section Roseum. The conidia show in side view hyperbolic or parabolic curves, in contrast to *Fusarium metacroum* App. and Wollenw., the conidia of which are as a rule more nearly straight. Blue globose sclerotia, 50–70μ thick, occur and form a striking contrast to the carmine plectenchymatic thallus on starchy media, such as steamed potato tubers. Both blue and carmine are basic modifications of the fungus, while yellow (on rice) is the acid one, turning blue to purple violet with the addition of an alkali.

" Habitat. Occurs on partly decayed plants, especially on stems, roots, and tubers, also on fruits. Found on Solanum, Ipomoea, Fagus (beech nuts), and *Impatiens balsamina* in the United States of America."

Ellis and Everhart's description is incomplete, but Wollenweber says (on page 270 of reference cited) that he " found this fungus so widely distributed on potato stems in the New England States that he feels justified in identifying it as *Fusarium acuminatum*." The writer did not study this fungus.

12. *Fusarium metacroum* Ap. et Wr. (Fig. 10; Pl. vii. fig. 5)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:132–141; Pl. i, figs. 111 to 118; Pl. iii, fig. 8. 1910.

Conidia broad, ellipsoid, more or less pointed at apex, seldom prominently pedicellate, typically 5–septate, 53 x 4.1 (43–65 x 3.8–4.3)μ, often 3– or 4–septate, seldom 0– to 2–, rarely 6–, exceptionally up to 12–septate, in minute, more or less converging, sporodochia forming exposed pseudopionnotes, from corinthian red to clay color, typically from dragon's-blood red to brick red; chlamydospores absent; aërial mycelium typically absent; substratum from madder brown and brazil red to russet color; conidia often densely granulate with indistinct septation.

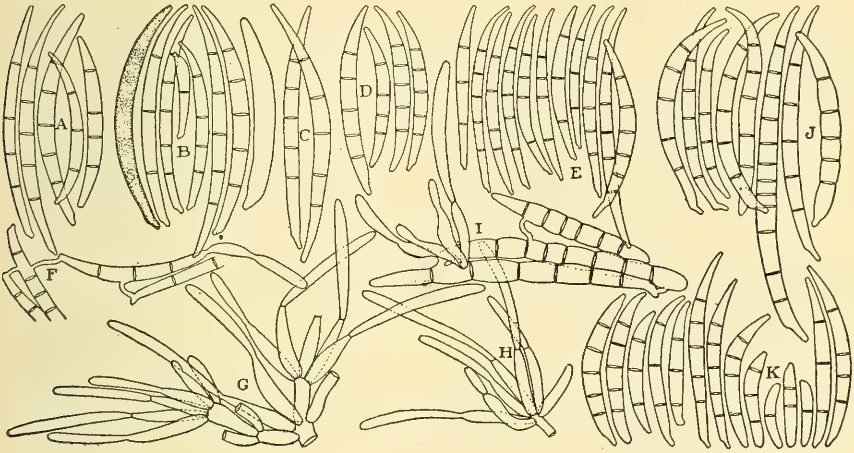

Fig. 10.—*Fusarium metacroum. Pseudopionnotal conidia:* a, *from 65-days-old culture on rye grain;* b, *from 10-days-old culture on hard lima-bean agar;* c, *from 8-days-old culture on hard lima-bean agar with 2 per cent glucose;* d, *from 113-days-old culture on potato stem plug;* e *and* f, *from 23-days-old culture on red raspberry cane plug;* i *and* j, *from 11-days-old culture on slightly acidified hard potato agar;* k, *from 66-days-old culture on rye grain.* g *and* h, *Conidiophores from 23-days-old culture on red raspberry cane plug*

Hab. On grains of *Triticum vulgaris* in Germany and on rotted tubers of *Solanum tuberosum* in New York State.

The organism was isolated, together with *F. diversisporum*, from a rotted tuber from Long Island. The two fungi were growing together, and in the original culture the mixture appeared to be a pink Fusarium. The fungi were separated by dilution and remained very distinct from each other. *F. metacroum* isolated by the writer is in all respects identical with the originally described organism.

The measurements of the conidia from the original culture when grown on various media are as follows:

On red raspberry cane plug, culture twenty-three days old:
Conidia: 3–septate, 42 per cent, 46 x 3.2 (35–56 x 2.6–4.1)μ
4–septate, 28 per cent, 50 x 3.5 (40–62 x 2.9–4.1)μ
5–septate, 30 per cent, 50 x 3.8 (45–63 x 3.3–4.3)μ

On hard lima-bean agar with 2 per cent glucose, culture eleven days old:
Conidia: 3–septate, 8 per cent
4–septate, 7 per cent
5–septate, 75 per cent, 54 x 4.1 (35–62 x 3.5–5.7)μ
6–septate and more, 10 per cent, the largest spore 12–septate, 91 x 5.3μ

On slightly acidified hard potato agar, culture eight days old:
Conidia: 3–septate, 15 per cent
4–septate, 15 per cent
5–septate, 70 per cent, 65 x 4.2 (55–73 x 3.8–4.8)μ

On potato tuber plug, culture ninety-eight days old:
Conidia: 1–septate, 5 per cent, 19 x 3 (14–21 x 2.7–3.5)μ
2–septate, few
3–septate, 10 per cent, 36 x 3.8 (28–43 x 3.5–4.2)μ
4–septate, 30 per cent, 46 x 3.9 (36–51 x 3.5–4.7)μ
5–septate, 55 per cent, 50 x 4 (42–63 x 3.5–4.4)μ

On potato stem plug, culture one hundred and thirteen days old:
Conidia: 3–septate, 25 per cent, 36 x 3.7 (33–39 x 3.4–3.9)μ
4–septate, 20 per cent, about 39 x 4μ (only a few measured)
5–septate, 55 per cent, 43 x 4 (38–47 x 3.5–4.2)μ, the longest 5–septate, 63 x 3.85μ

On whole steamed potato tuber, culture thirty-eight days old:
Conidia: 3–septate, 40 per cent, 42 x 3.9 (37–53 x 3.5–4.1)μ
4–septate, 35 per cent, 45 x 4.1μ
5–septate, 25 per cent, 54 x 4.3 (47–63 x 3.7–4.8)μ

On rye grain, culture sixty-five days old:
(1) From a slimy heap of conidia 1 millimeter in diameter
Conidia: 3–septate, 27 per cent, 43 x 3.8 (31–49 x 2.9–4.7)μ
4–septate, 11 per cent, 44 x 4.1 (40–63 x 2.6–4.8)μ
5–septate, 62 per cent, 54.5 x 4.1 (43–67 x 3–5.2)μ
(2) From a minute, semi-dry fleck of conidia, culture sixty-six days old
Conidia: 0–septate, 2 per cent

$$\left.\begin{array}{l}\text{1–septate, 3 per cent, } 20 \times 3.5\\ \text{2–septate, 3 per cent, } 23 \times 3.9\end{array}\right\}\text{(only a few measured)}$$

3–septate, 65 per cent, 32 x 3.9 (22–47 x 3.5–4.4)μ
4–septate, 12 per cent, 43 x 4.1 (38–48 x 3.5–4.4)μ
5–septate, 15 per cent, 45 x 4.3 (38–48 x 3.5–4.7)μ, the largest 53 x 5.25μ

On hard lima-bean agar, culture ten days old:
Conidia: 3–septate, 6 per cent, 48 x 3.7μ (only three measured)
4–septate, 4 per cent
5–septate, 90 per cent, 61 x 3.9 (43–68 x 3.5–4.1)μ

Average of the above measurements:
Conidia: 0– to 2–septate, rare
3–septate, 26 per cent, 40.4 x 3.7μ
4–septate, 19 per cent
5–septate, 54 per cent, 53 x 4.1μ
6– and 7–septate, 1 per cent

The averages of Appel and Wollenweber's measurements are as follows:
Conidia: 3–septate, 9 per cent, 39 x 3.9μ
4–septate, 14 per cent, 43 x 4.2μ
5–septate, 76 per cent, 53 x 4.3μ
6–septate, 1 per cent, 63 x 4.6μ

13. *Fusarium metacroum* Ap. et Wr. var. **minus** n. var. (Fig. 11)

Conidial type as in *F. metacroum*, 5–septate conidia 54 x 3.6 (46–60 x 3.4–3.9)μ.

Hab. On stem of *Solanum tuberosum*, New York State.

Differs from *F. metacroum* by narrower conidia, often distinct plectenchymic substratum, and swellings in hyphæ very similar to true chlamydospores.

The organism was isolated only once, from a half-dead stem of potato plant at Atlanta, New York, from a pseudopionnotal spore mass. The measurements of the conidia produced by the organism on various media are as follows:

On red raspberry cane plug, culture thirty-one days old:

Conidia: 1–septate, 0.5 per cent, 20 x 2.5μ
2–septate, very few
3–septate, 46.5 per cent, 43 x 2.8 (23–53 x 2.3–3.5)μ
4–septate, 31 per cent, 48 x 3.2 (40–60 x 2.3–3.5)μ
5–septate, 22 per cent, 52 x 3.4 (42–60 x 2.9–4)μ
6–septate, rare, 61 x 3.4μ (only one measured)

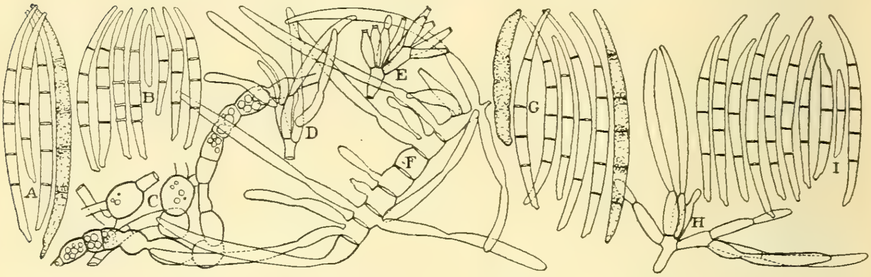

Fig. 11.—*Fusarium metacroum var. minus.* A, *Conidia from 18-days-old culture on hard lima-bean agar;* B, *conidia from the original specimen, potato stem, August 7, 1912;* C, *chlamydospore-like structures of hyphæ from 26- and 176-days-old cultures on corn agar;* D, *conidiophore from the original specimen, potato stem, August 7, 1912;* E, *conidiophores from 31-days-old culture on red raspberry cane plug;* F, *conidia producing numerous conidiophores from 10-days-old culture on hard lima-bean agar;* G, *normal conidia from 10-days-old culture on hard lima-bean agar;* H, *conidiophores from 31-days-old culture on red raspberry cane plug;* I, *conidia from 31-days-old culture on red raspberry cane plug. All conidia are from pseudopionnotes*

On hard lima-bean agar with 2 per cent glucose, culture eight days old:

Conidia: 3–septate, 17 per cent
4–septate, 17 per cent
5–septate, 66 per cent, 60 x 3.6 (53–65 x 3–4.2)μ

On potato tuber agar, culture ninety-eight days old:

Conidia: 0–septate, 5 per cent
1–septate, 5 per cent
2–septate, rare
3–septate, 20 per cent, 34 x 3.5 (26–39 x 3–4)μ
4–septate, 30 per cent, 42 x 3.8 (36–48 x 3.5–4.1)μ
5–septate, 40 per cent, 46 x 3.9 (40–54 x 3.5–4.1)μ

On rye grain, culture sixty-five days old:
Conidia: 3–septate, 20 per cent, 41 x 3.2 (36–46 x 2.9–4)μ
4–septate, 17 per cent, 48 x 3.3 (40–54 x 2.9–4)μ
5–septate, 63 per cent, 52 x 3.7 (40–60 x 3–4.1)μ

On hard lima-bean agar, culture ten days old:
Conidia: 1–septate, very few
3–septate, 11 per cent, 50 x 3.3 (29–61 x 3–3.7)μ
4–septate, 4 per cent
5–septate, 85 per cent, 60 x 3.5 (45–67 x 3.1–4)μ

Average of the above measurements:
Conidia: 0– to 2–septate, 2 per cent, 20 x 2.5μ
3–septate, 23 per cent, 42 x 3.2μ
4–septate, 20 per cent
5–septate, 55 per cent, 54 x 3.6μ
6–septate, very rare, 61 x 3.4μ

14. *Fusarium subulatum* Ap. et Wr. (Fig. 1w; Fig. 12, G to J; Pl. II, fig. 11; Pl. VII, fig. 4)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:118–132, Pl. II, figs. 65 to 87. 1910. Wollenweber, H. W., Phytopath. **3**:32, fig. 1N. 1913.

Conidia slightly elliptically curved, typically of nearly even diameter for the greater part of their length, very gradually attenuate toward both ends, slightly pedicellate, typically 5–septate, 58 x 3.64 (48–65 x 3.4–3.85)μ, usually in numerous, sometimes converging sporodochia ($\frac{1}{4}$ to 1 millimeter in diameter), produced near substratum and lower aërial mycelium, from pink-flesh to apricot-buff and from coral red to brick red in color, with darker shades in old moist cultures; chlamydospores absent; aërial mycelium typically present, at first hyaline, then testaceous color and other hues of red; on various agars from vinaceous tawny and madder brown to pomegranate purple and Eugenia red.

Hab. A cosmopolitan saprophyte, of very wide occurrence on dead substrata, in soil and water, also parasitic on cereals and on tubers of *Solanum tuberosum*.

The organism was twice isolated by the writer from rotted potato tubers, and the strains were compared with a culture of the originally described organism obtained through the courtesy of Dr. Wollenweber and proved to be identical. The writer's measurements of conidia of the original strain are as follows:

On red raspberry cane plug, culture twenty-seven days old:
Conidia: 3–septate, 10 per cent, 55 x 2.9μ
4–septate, 20 per cent, 60.4 x 3 (54–76 x 2.6–3.5)μ
5–septate, 70 per cent, 65 x 3.4 (57–76 x 2.6–4.1)μ

On potato tuber plug, culture ninety-nine days old:
Conidia: 0– to 2–septate, none to very few
3–septate, 35 per cent, 33 x 3.7 (27–40 x 3–4)μ
4–septate, 20 per cent, 38 x 3.7 (32–42 x 3–4.1)μ
5–septate, 45 per cent, 48 x 3.85 (42–58 x 3.5–4.1)μ
6–septate, rare, 57 x 4μ (only one measured)

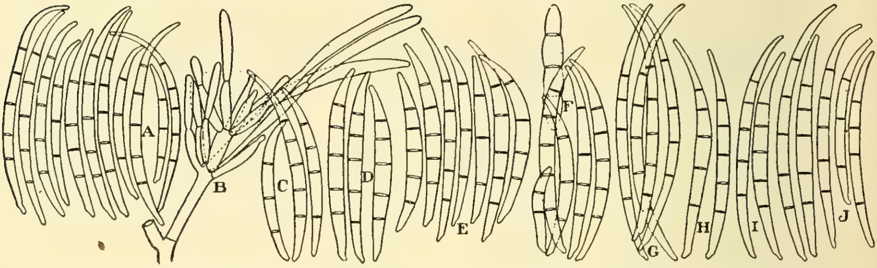

Fig. 12.— A-F, *Fusarium subulatum var. brevius:* A, *Conidia,* B, *conidiophore, from small sporodochium of 31-days-old culture on red raspberry cane plug;* C, *sporodochial conidia from 113-days-old culture on potato stem plug;* D, *pseudopionnotal conidia from 38-days-old culture on whole steamed potato tuber;* E, *sporodochial conidia from 65-days-old culture on rye straw;* F, *aërial conidia from 10-days-old culture on hard lima-bean agar.*

G-J, *F. subulatum:* G, *Sporodochial conidia from 31-days-old culture on red raspberry cane plug;* H, *semi-dry conidia from sporodochium of 38-days-old culture on steamed potato tuber;* I, *sporodochial conidia from 113-days-old culture on potato stem plug;* J, *sporodochial conidia from 65-days-old culture on rye grain*

On potato stem plug, culture one hundred and thirteen days old:
Conidia: 3–septate, 5 per cent, about 35 x 3μ (only a few measured)
4–septate, 4 per cent
5–septate, 91 per cent, 54 x 3.6 (47–65 x 3.2–3.9)μ

On steamed potato tuber, culture thirty-eight days old:
Conidia: 3–septate, 3 per cent, about 40 x 3.5μ
4–septate, 8 per cent, about 48 x 3.6μ
5–septate, 89 per cent, 55 x 3.7 (37–63 x 3.5–3.9)μ
6–septate, few, same measurement as 5–septate

On rye grain, culture sixty-five days old:

Conidia: 3–septate, 4 per cent, 52 x 3.4 (42–55 x 2.9–4)μ
4–septate, 15 per cent, 54 x 3.5 (42–67 x 2.9–4.1)μ
5–septate, 81 per cent, 64 x 3.7 (45–75 x 2.9–4.2)μ

On hard lima-bean agar, culture ten days old:

Conidia: 0–septate, rare
2–septate, very rare
3–septate, 11 per cent, 45 x 3.1 (33–60 x 3–3.5)μ
4–septate, 19 per cent
5–septate, 70 per cent, 60 x 3.6 (53–67 x 3.1–4)μ
9–septate, rare, 69 x 5.7μ (only one measured)

Average of the above measurements:

Conidia: 0–septate, none to rare
1– and 2–septate, none to rare
3–septate, 11 per cent, 43.3 x 3.3μ
4–septate, 15 per cent
5–septate, 74 per cent, 58 x 3.64μ
6– to 9–septate, none to rare

The averages of Appel and Wollenweber's measurements for the same organism are as follows:

Conidia: 1–septate, rare

$$\left.\begin{array}{l}\text{3–septate}\\ \text{4–septate}\end{array}\right\}\text{28 per cent}\left\{\begin{array}{l}36.5 \text{ x } 3.86\mu\\ 51.5 \text{ x } 3.5\mu\end{array}\right.$$

5–septate, 62 per cent, 61 x 3.86μ
6–septate, 9 per cent, 69 x 4μ
7–septate, 1 per cent

This shows the 5–septate conidia to be in about the same proportion and of about the same size as found by the writer.

15. *Fusarium subulatum* Ap. et Wr. var. **brevius** n. var. (Fig. 12, A to F; Pl. II, fig. 12; Pl. VII, fig. 3)

Conidia and fruiting forms of the same type as those of *F. subulatum;* chlamydospores also absent; 5–septate conidia average 50 x 3.8 (41–58 x 3.1–4.2)μ in size.

Hab. On rotted tuber of *Solanum tuberosum*, Ithaca, New York.

Differs from *F. subulatum* Ap. et Wr. mainly in shorter conidia, absence of carmine color in substratum, and high, better-developed, aërial mycelium.[39]

The measurements of the conidia on various media are as follows:

On red raspberry cane plug, culture thirty-one days old:

Conidia: 3–septate, 35 per cent, 40 x 2.7 (29–49 x 2.3–3.3)μ
4–septate, 20 per cent, 51 x 3 (45–53 x 2.3–3.4)μ
5–septate, 45 per cent, 55 x 3.1 (48–60 x 2.9–3.8)μ

On potato tuber plug, culture ninety-nine days old (in general, spores more or less deteriorated):

Conidia: 1–septate, 1 per cent
3–septate, 33 per cent, 30 x 3.6 (21–42 x 3–4)μ
4–septate, 20 per cent, 36 x 3.9 (33–42 x 3.5–4.1)μ
5–septate, 46 per cent, 41 x 4 (35–48 x 3.5–4.1)μ
6–septate, rare, 43 x 4.1μ (only one measured)

On potato stem plug, culture one hundred and thirteen days old:

Conidia: 3–septate, 67 per cent, 34 x 3 (28–49 x 2.9–3.6)μ
4–septate, 20 per cent, 42 x 3.3 (37–48 x 3–3.8)μ
5–septate, 13 per cent, 44.6 x 3.4 (38–47 x 3–3.8)μ

On whole steamed potato tuber, culture thirty-eight days old:

Conidia: 3–septate, 25 per cent, 37 x 3.8 (31–42 x 3.5–4)μ
4–septate, 35 per cent, 43 x 4 (36–49 x 3.5–9.2)μ
5–septate, 40 per cent, 47 x 4.1 (40–56 x 3.5–4.4)μ
6–septate, few, 52 x 4.4 (only a few measured)

On rye grain, culture sixty-five days old:

Conidia: 3–septate, 16 per cent, 42 x 4 (36–49 x 3.5–4.3)μ
4–septate, 17 per cent, 45 x 4.1 (36–54 x 3.5–4.3)μ
5–septate, 67 per cent, 51 x 4.2 (43–58 x 3.7–4.7)μ

On hard lima-bean agar, culture ten days old:

Conidia: 0–septate, very rare
1–septate, very rare
2–septate, very rare
3–septate, 24 per cent, 41 x 3.3 (36–48 x 3–3.5)μ
4–septate, 20 per cent
5–septate, 56 per cent, 52 x 3.9 (42–59 x 3.2–4.1)μ

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[39] This grows straight up and out from the point of inoculation in a plate culture on potato agar in the first week of its growth, while the aërial mycelium in *F. subulatum* is always of a more or less loose, feltlike character, uniformly medium short over the surface of the colony.

On medium potato agar, culture ten days old:

Conidia: 3–septate, 9 per cent, about 45 x 3.3 (only two measured)
4–septate, 3 per cent
5–septate, 88 per cent, 58 x 3.5 (47–64 x 3.1–4)μ

Average of the above measurements:

Conidia: 0– to 2–septate, absent or more or less rare
3–septate, 30 per cent, 38.4 x 3.4μ
4–septate, 19 per cent
5–septate, 51 per cent, 49.8 x 3.76μ
6–septate, absent to 1 per cent, 47.5 x 4.25μ

16. **Fusarium effusum** n. sp. (Fig. 13; Pl. VII, fig. 6)

Conidia gradually pointed toward apex, distinctly but not prominently pedicellate, typically 5–septate, 50 x 4.3 (44.5–57 x 3.9–4.5)μ, often 3– to 7–, seldom 1– to 2–septate; single or in indistinct pseudopionnotes and in large (on oats, wheat, and the like, about ½ centimeter in diameter) plectenchymic sporodochia; shape of conidia of nearly the same type, in all stages and in mass, of salmon color and its tints; aërial mycelium typically well developed, though on an agar may be resupinate, uniform, without any differentiated tufts or strands, from white to cream and tints of salmon color; substratum, on glucose agar, from chamois to morocco red, and on glucose-free agar, from colorless to Eugenia red; typical spore germination by straight, unbranched tubes (Fig. 13 J); mycelium in young colony (Fig. 13 H) typically composed of nearly straight, sparse in number, and more or less regular, branches.

Hab. On dry tubers of *Solanum tuberosum*, Minnesota.

Latin description.—Conidiis gradatim in apicem acutis, distincte sed non insignite pedicellatis, typice 5–septatis, 50 x 4.3 (44.5–57 x 3.9–4.5)μ, saepe 3–7–, raro 1–2–septatis; continuis vel in indistinctis pseudopionnotibus atque in magnis (in avena, tritico, etc., circa ½ cm. diam.) plectenchymicis sporodochiis; conidiis prope eodem in omnibus gradibus typo, in totum "salmon-color" (R); aerio mycelio typice plene maturo, sed in agare interdum resupinato, uniformi, sine ullis discretis cristis aut fibris, ex albo "cream color" (R) "salmon color" (R) vel simili colore; substrato in agare glucoso e "chamois" (R) "morocco red" (R), in agare non glucoso ex hyalino "Eugenia red" (R); sporis per rectos et non ramosos cylindros typice germinatis (Fig. 13 J); mycelio in colonia juveni ex prope rectis, sparsis, plus minusve regularibus ramis composito.

Hab. In tuberibus aridis Solani tuberosi, Minnesota, Amer. bor.

The fungus was isolated in association with *F. Solani* from an old rotted potato tuber from Minnesota. Measurements of conidia from cultures on different media are as follows:

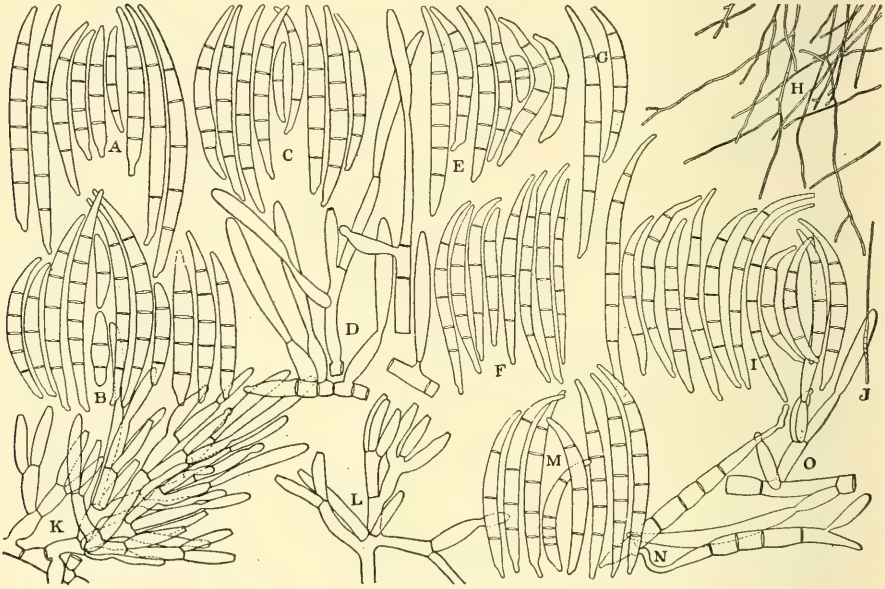

Fig. 13.—*Fusarium effusum.* A, *Pseudopionnotal conidia from 7-days-old culture on hard lima-bean agar with 2 per cent glucose;* B, *pseudopionnotal conidia from 13-days-old culture on hard lima-bean agar (rare forms of conidia shown at the center);* C, *pseudopionnotal conidia,* D, *conidiophores, from 9-days-old culture on medium potato agar;* E, *sporodochial conidia from 24-days-old culture on potato tuber plug;* F, *sporodochial conidia from 76-days-old culture on red raspberry cane plug;* G, *typical sporodochial conidia from 107-days-old culture on potato stem plug;* H, *character of colony growth (magnification 80 times),* I, *sporodochial conidia, from 34-days-old culture on rye grain;* J, *character of spore germination from potato-decoction hanging drop in van Tieghem cell (magnification 80 times);* K, L, *conidiophores,* M, *normal conidia,* N, *anastomosed conidia,* O, *conidiophores, from 36-days-old culture on corn meal*

On slightly acidified hard potato agar, culture ten days old; conidia from pseudopionnotes:

Conidia: 2–septate, rare
3–septate, 50 per cent, 34 x 4.1 (28–47 x 3–4.7)μ
4–septate, 10 per cent, 45 x 4.2μ
5–septate, 40 per cent, 50 x 4.5 (42–65 x 3.5–5.3)μ

On red raspberry cane plug, culture seventy-six days old; conidia from a sporodochium:

Conidia: 3–septate, 35 per cent, 42 x 3.3 (32–48 x 2.7–4)μ
4–septate, 15 per cent, 47 x 3.6 (42–53 x 3.2–4.3)μ
5–septate, 50 per cent, 49 x 3.9 (38–58 x 3.2–4.4)μ
6–septate, very rare

On hard lima-bean agar, 2 per cent glucose, culture seven days old; conidia from mycelium:

Conidia: 1–septate, 1 per cent
2–septate, 5 per cent
3–septate, 19 per cent, 39 x 4.2 (28–50 x 3.9–4.8)μ
4–septate, 10 per cent
5–septate, 60 per cent, 57 x 4.3 (52–72 x 4–5.3)μ
6–septate, 5 per cent, 62 x 4.7 (49–72 x 4.3–5.3)μ
7–septate, rare, 72 x 4.3μ (only one measured)
8–septate, exceptional, 87.5 x 5.8μ (only one measured)

On potato tuber plug, culture twenty-four days old; conidia from a sporodochium:

Conidia: 0–septate
1–septate, 2 per cent
2–septate, 1 per cent
3–septate, 12 per cent, 32 x 4.3 (28–36 x 3.9–4.4)μ
4–septate, 21 per cent, 34 x 4.5 (29–41 x 4.1–4.7)μ
5–septate, 61 per cent, 44.5 x 4.5 (35–51 x 4.1–4.8)μ
6– and 7–septate, 3 per cent, 53 x 4.7 (49–54 x 4.3–5.2)μ

On potato stem plug, culture one hundred and seven days old; conidia from a sporodochial mass (spores mostly with deteriorated ends):

Conidia: 3–septate, 5 per cent
4–septate, 15 per cent
5–septate, 80 per cent, 49 x 4 (35–63 x 3.5–4.7)μ

On hard lima-bean agar, culture ten days old; conidia from aërial mycelium:

Conidia: 1–septate, rare, 21 x 3.1μ (only one measured)
3–septate, 11 per cent, 36.2 x 4 (28–42 x 3.1–4.8)μ

4–septate, 4 per cent
5–septate, 81 per cent, 50.5 x 4.5 (38–60 x 3.9–4.8)μ
6–septate, 4 per cent, 56 x 4.7 (48–64 x 4.4–5.2)μ
7–septate, rare, 62 x 5.2μ (only one measured)

On the same medium as above, culture sixteen days old; conidia from aërial mycelium and indistinct pseudopionnotes:

Conidia: 1–septate, 1 per cent, 18 x 3.5μ (only a few measured)
2–septate, 1 per cent, 24 x 4μ (only a few measured)
3–septate, 12 per cent, 33 x 4 (21–44 x 2.9–4.8)μ
4–septate, 6 per cent
5–septate, 71 per cent, 50 x 4.3 (38–62 x 3.5–4.7)μ
6–septate, 7 per cent, 60 x 4.7 (55–64 x 4.5–4.8)μ
7–septate, 2 per cent, 62 x 4.7 (59–65 x 4.4–4.8)μ

Average of the above measurements:

Conidia: 1–septate, less than 1 per cent, 18 x 3.5μ
2–septate, 2 per cent, 24 x 4.0μ
3–septate, 20 per cent, 36 x 4.0μ
4–septate, 12 per cent
5–septate, 63 per cent, 50 x 4.3μ
6– and 7–septate, 3 per cent, 68 x 4.8μ

F. effusum, especially in its sporodochial stage, much resembles the following other species: *F. subulatum, F. lucidum, F. biforme, F. diversisporum.* From *F. subulatum* it can be at once distinguished by the diameter of the conidia and by the larger size of sporodochia.[40] From *F. lucidum* it differs primarily by the common presence of 6– and 7–septate conidia, which are absent in *F. lucidum,* and by denser red substratum than that of the latter species. From *F. biforme* it differs mainly by absence of the long, 9– or more septate, conidia in pseudopionnotes, and also by absence of arthrosporial conidial form, the pluriseptate conidia and arthrosporial conidia on aërial mycelium being more or less common in *F. biforme.* From *F. diversisporum* it differs by absence of arthrosporial conidia, which are typically produced on aërial mycelium of *F. diversisporum.*

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[40] Sporodochia of *F. subulatum* as a rule are small, but on whole steamed potato tubers they may be as large as those of *F. effusum.*

17. Fusarium truncatum n. sp. (Figs. 1, C_1 to E_1, and 14; Pl. VII, fig. 1)

Conidia typically sickle-shaped, gradually pointed toward the apex, slightly broader at or just above the middle, distinctly pedicellate, 3– to 5–septate; 3–septate averaging 35 x 3.7 (31.5–42 x 3.4–3.9)μ, 5–septate averaging 45 x 3.9 (43–48 x 3.4–4.2)μ; from cinnamon and terra cotta to carmine-pomegranate purple in color; conidiophores from loose to dense, bushlike, single or in from small to large (up to $\frac{1}{2}$ centimeter in diameter) sporodochia; aërial mycelium always well developed, mostly composed of fine but macroscopically distinct threads, from white to slightly carmine near substratum; color of substratum, on hard potato agar rich in glucose, varies from pale cinnamon and diffuse salmon hues to corinthian and brick red, more or less distinctly zonate.

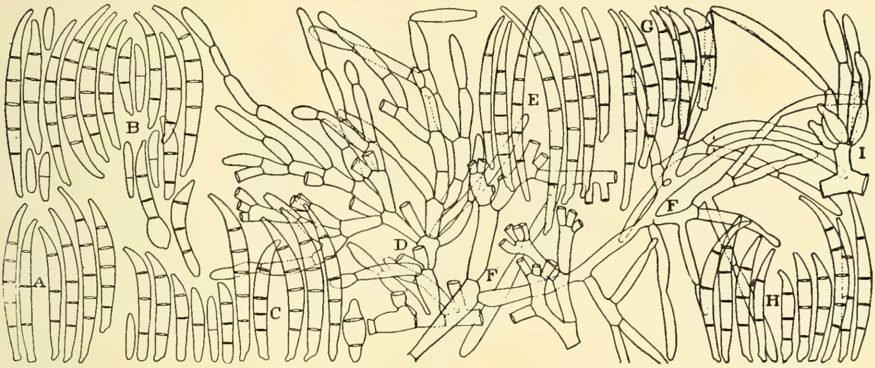

FIG. 14.—*Fusarium truncatum.* A, *Sporodochial conidia from 60-days-old culture on red raspberry cane plug;* B, *aërial conidia from 13-days-old culture on hard potato agar;* C, *aërial conidia (some with distinctly truncate basal cell) from 14-days-old culture on medium potato agar;* D, *conidiophore from 13-days-old culture on hard potato agar;* E, *sporodochial conidia from 14-days-old culture on medium potato agar;* F, *conidiophores from 47-days-old culture on potato tuber plug;* G, *sporodochial conidia from 37-days-old culture on hard oat agar;* H, *conidia from aërial mycelium from 47-days-old culture on potato tuber plug;* I, *conidiophore from 4-days-old colony in petri dish on hard potato agar with 10 per cent glucose*

Hab. On rotted tubers of *Solanum tuberosum*, New York State.

Latin description.—Conidiis typice falciformibus, gradatim in apicem acutis, paulo latioribus medio vel subinde supra medium, distincte pedicellatis 3–5–septatis; 3–septatis plerumque 35 x 3.7 (31.5–42 x 3.4–3.9)μ, 5–septatis plerumque 45 x 3.9 (43–48 x 3.4–4.2)μ; e "cinnamon" (R) et "terra cotta" (R) "carmine" (R) et "pomegranate purple" (R); conidiophoris laxis demum dense, fruticosis, continuis vel in parvis magnisve (usque ad ½ cm. diam.) sporodochiis; aerio mycelio semper plene maturo, ex hyphis subtilibus sed macroscopice distinctis composito, ex albo paulum "carmine" (R) prope substratum; substrato in duro agare glucoso Solani tuberosi e pallide "cinnamon" (R) et "salmon color" (R) diffuse "corinthian" et "brick red" (R), plus minusve distincte zonato.

Hab. In tuberibus putridis Solani tuberosi, New York, Amer. bor.

Measurements of conidia from cultures on different media are as follows:

On slightly acidified hard potato agar, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 7 per cent
1–septate, 54 per cent
2–septate, 6 per cent
3–septate, 22 per cent, 31.5 x 3.75 (21–45 x 3.1–4.1)μ
4–septate, 4 per cent, 40 x 4.1 (32–49 x 3.5–4.7)μ
5–septate, 7 per cent, 43 x 4.1 (33–53 x 3.9–4.7)μ
6–septate, rare, 45 x 4.5μ (only two measured)

On potato tuber plugs, culture forty-six days old; conidia from a sporodochium 2 millimeters in diameter:

Conidia: 3–septate, 63 per cent, 37 x 3.4 (27–49 x 3–3.6)μ
4–septate, 22 per cent, 41 x 3.7 (40–45 x 3.5–4)μ
5–septate, 15 per cent, 43 x 3.7 (40–45 x 3.5–4.1)μ

On red raspberry cane plug, culture sixty days old; conidia from a sporodochium about 2 millimeters in diameter:

Conidia: 3–septate, 29 per cent, 37 x 3.9 (28–41 x 3.5–4.2)μ
4–septate, 31 per cent, 40 x 3.9 (35–43 x 3.5–4.2)μ
5–septate, 40 per cent, 44 x 4 (36–46 x 3.5–4.5)μ

On potato stem plug, culture eighty-three days old; conidia from a sporodochium about 1½ millimeters in diameter (many of the conidia much deteriorated):

Conidia: 3–septate, 20 per cent
4–septate, 16 per cent
5–septate, 64 per cent, 48 x 3.4 (44–51 x 3.1–3.7)μ

On soft potato agar, plate culture fourteen days old; conidia from thick pseudopionnotal mass near the inoculation point:

Conidia: 3–septate, 20 per cent, 42 x 3.4 (35–51 x 3–3.9)μ
4–septate, 13 per cent
5–septate, 67 per cent, 50 x 3.6 (43–65 x 3.1–3.9)μ

On same medium as above, plate culture also; conidia from a small sporodochium on aërial mycelium:

Conidia: 0–septate, rare
1–septate, 3 per cent, 16 x 3.1μ (only one measured)
2–septate, 2 per cent, 21 x 3.5μ (only one measured)
3–septate, 29 per cent, 33 x 3.8 (22–41 x 3.3–4.2)μ
4–septate, 19 per cent
5–septate, 47 per cent, 45 x 4.2 (36–64 x 4–4.4)μ

Average of the above measurements:

Conidia: 0–septate, about 1 per cent
1–septate, about 9 per cent
2–septate, about 1 per cent
3–septate, about 31 per cent, 34.8 x 3.7μ
4–septate, about 18 per cent
5–septate, about 40 per cent, 45 x 3.9μ
6–septate, very exceptional, 45 x 4.5μ (only two measured)

Conidia of *F. truncatum* often have a peculiar flat base, and this species can be separated from all the other Fusaria of potatoes by its typically (though not always) pomegranate purple to carmine conidial masses.

18. **Fusarium lucidum** n. sp. (Figs. 1v and 15; Pl. II, figs. 9 and 10; Pl. IV, fig. 12; Pl. VI, fig. 12)

Conidia typically ellipsoid, very gradually attenuate toward both ends, distinctly but not prominently pedicellate, 5–septate, 54 x 4.05 (43–63 x 3.7–4.7)μ, salmon, often of very bright hues, but paler or denser in from small to large (up to ½ centimeter in diameter) sporodochia; no chlamydospores; conidiophores typically more or less compound, bushlike; mycelium from white to pale cinnamon and pomegranate near substratum; when first isolated the fungus has a substratum, on potato agar without glucose from pale pink to tints of pomegranate, and on the same medium with glucose from clay to buckthorn brown.

Hab. On rotted tubers of *Solanum tuberosum*, New York State.

Differs from *F. effusum* mainly by absence or very rare occurrence of 6– and 7–septate conidia.

Latin description.—Conidiis typice ellipsoidalibus, maxime in utrosque terminos gradatim attenuatis, distincte sed non insignite pedicellatis, 5–septatis, 54 x 4.05 (43–63 x 3.7–4.7)μ, "salmon-color" (R) magnis, in parvis (usque ad $\frac{1}{2}$ cm. diam.) sporodochiis; nullis chlamydosporis; conidiophoris typice plus minusve compositis, fruticosis; mycelio ex albo "pale cinnamon" (R) vel "pomegranate" (R) prope substratum; primum substrato, in agare Solani tuberosi non glucoso, e pallide rubello "pomegranate" (R) vel simili colore, in eodem agare glucoso, ex argillaceo "buckthorn-brown" (R).

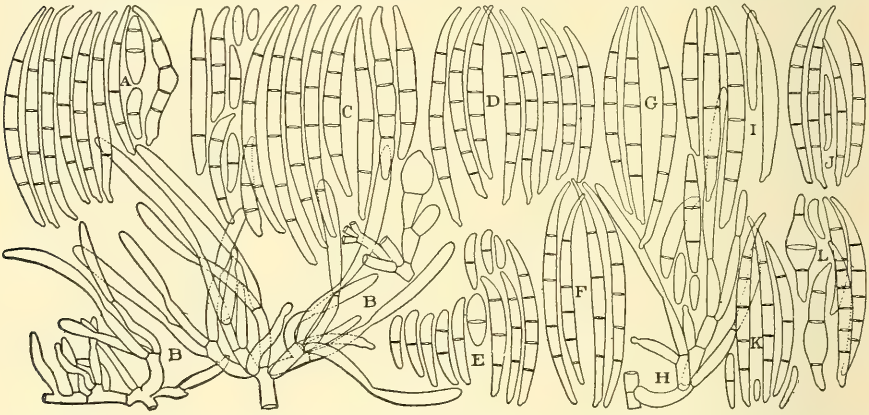

Fig. 15.—*Fusarium lucidum.* a, *Sporodochial conidia,* b, *conidiophores, from 47-days-old culture on wheat kernels;* c, *pseudopionnotal conidia from 11-days-old culture on slightly acidified hard potato agar;* d, *sporodochial,* e, *aërial, conidia from 75-days-old red raspberry cane plug;* f, *pseudopionnotal conidia from 36-days-old culture on hard oat agar;* g, *sporodochial conidia,* h, *conidiophore, from 20-days-old culture on hard lima-bean agar with 2 per cent glucose;* i, *pseudopionnotal conidia from 7-days-old culture on hard lima-bean agar with 2 per cent glucose;* j, *sporodochial conidia from 65-days-old culture on potato stem plug;* k, *sporodochial conidia from 24-days-old culture on potato tuber plug;* l, *sporodochial conidia from 82-days-old culture on potato tuber plug (the two at the left abnormal)*

Hab. In tuberibus putridis Solani tuberosi, New York, Amer. bor.

Measurements of conidia from cultures on different media are as follows:

On hard potato agar, slightly acidified, culture eleven days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, 11 per cent, 17 x 3.1 (12–22 x 2.9–3.5)μ
2–septate, 7 per cent, 28 x 3.8 (20–32 x 2.9–4.3)μ
3–septate, 68 per cent, 43.7 x 3.8 (33–67 x 3–4.4)μ
4–septate, 4 per cent, 55 x 4.2 (47–65 x 4–4.4)μ
5–septate, 10 per cent, 63 x 4.4 (57–72 x 4–4.8)μ

On red raspberry cane plug, culture seventy-five days old; conidia from aërial mycelium:

Conidia: 0–septate, rare
1–septate, 1 per cent
3–septate, 5 per cent, 43 x 3.8 (25–48 x 3.5–4.4)μ
4–septate, 14 per cent, 45 x 3.8 (38–54 x 3–4.6)μ
5–septate, 80 per cent, 51 x 4.2 (42–60 x 3.5–4.7)μ
6–septate, rare

On potato tuber plugs, culture ninety-two days old; conidia from a mass of minute sporodochia:

Conidia: 0–septate, rare
1–septate, 3 per cent
2–septate, 2 per cent
3–septate, 25 per cent, 31 x 3.8 (24–36 x 3–4.1)μ
4–septate, 17 per cent
5–septate, 53 per cent, 46 x 3.9 (36–58 x 3.5–4.7)μ
6–septate, rare, 55–60 x 3.8–4.7μ (only a few measured)

On hard lima-bean agar with 2 per cent glucose, culture seven days old; conidia from aërial mycelium:

Conidia: 0–septate, 40 per cent
1–septate, 35 per cent
2–septate, 5 per cent
3–septate, 14 per cent, 41 x 4.2 (33–44 x 3.5–4.8)μ
4–septate, 5 per cent, 48 x 4.6 (42–53 x 4–5.3)μ
5–septate, 1 per cent, 56 x 4.7 (53–58 x 4.3–4.9)μ (only five spores measured, the largest 60 x 5.7μ)

On the same medium as above, culture twenty days old; conidia from a small sporodochium:

Conidia: 3–septate, 1 per cent
4–septate, 12 per cent, 44 x 4 (40–57 x 3.5–4.4)μ
5–septate, 87 per cent, 54 x 3.9 (40–68 x 3.5–4.2)μ

On potato tuber plug, culture twenty-four days old; conidia from aërial mycelium:

Conidia: 3–septate, 10 per cent, 46 x 3.4μ (only a few measured)
4–septate, 30 per cent, 47 x 3.5 (44–50 x 3–3.8)μ
5–septate, 60 per cent, 51 x 3.7 (48–57 x 3.4–4.2)μ
6–septate, rare, 60 x 4.2μ (only one measured)

On potato stem plug, culture one hundred and seven days old; conidia from a sporodochial mass (much deterioated):

Conidia: 3–septate, 15 per cent
4–septate, 5 per cent
5–septate, 80 per cent, 56 x 3.8 (50–63 x 3.5–4.2)μ

On steamed whole potato tuber, culture forty-nine days old; conidia from a white, semi-dry, sporodochial mass:

Conidia: 3–septate, 50 per cent, 36 x 3.9 (24–43 x 3.5–4.1)μ
4–septate, 35 per cent
5–septate, 15 per cent, 43 x 4.1 (39–46 x 3.9–4.4)μ

On same medium as above; conidia from a red (old?) sporodochial mass, nearly converging into semi-pionnotal layer:

Conidia: 0–septate, very rare
3–septate, 8 per cent, 46 x 3.7 (24–50 x 3–4)μ
4–septate, 12 per cent, 48 x 3.8 (41–52 x 3.5–4.1)μ
5–septate, 80 per cent, 54 x 4 (43–62 x 3.5–4.3)μ
6–septate, very rare, about 57 x 4.2μ (only a few measured)

On medium soft potato agar, culture fourteen days old; conidia from pseudopionnotes:

Conidia: 3–septate, 28 per cent, 42 x 3.6μ (only four measured)
4–septate, 10 per cent
5–septate, 62 per cent, 58 x 4 (43–65 x 3.5–4.7)μ

On hard lima-bean agar, culture fourteen days old; conidia from a sheet of small sporodochial masses spread over the substratum:

Conidia: 3–septate, 3 per cent, 41 x 3.2μ (only three measured)
4–septate, 1 per cent
5–septate, 96 per cent, 60 x 3.8 (52–70 x 3.5–5)μ

On wheat grain culture fourteen days old; conidia from small, semi-dry sporodochia:

Conidia: 1–septate, rare
3–septate, 14 per cent
4–septate, 26 per cent
5–septate, 60 per cent, 56 x 3.7 (49–64 x 3.3–4.1)μ

Average of the above measurements:

Conidia: 0–septate, about 3 per cent
1–septate, about 4 per cent, 17 x 3.1μ
2–septate, about 1 per cent
3–septate, about 20 per cent, 41 x 3.7μ
4–septate, about 14 per cent
5–septate, about 58 per cent, 54 x 4.05μ
6–septate, very rare, 58 x 4.2μ

VI. SECTION **ARTHROSPORIELLA** n. sec.

Microconidia short and broad, spindle-shaped, 0– to 3–septate[41]; sporodochial macroconidia when present sickle-shaped, mostly 5–septate, of Roseum type; pseudopionnotal microconidia mostly 5– and 5– to 7–, often to 9– and more, septate, from slightly curved to straight and anguiform; true chlamydospores absent; aërial mycelium from white to pale buff and different hues of red and pink; color of substratum from clay to different hues of red.

The section is a connecting link between sections Roseum and Sporotrichiella (through *F. arthrosporioides*).

19. **Fusarium diversisporum** n. sp. (Fig. 16; Pl. VII, fig. 12)

Conidia varying from arthrosporial (short, spindle-shaped, and having an average measurement when 3–septate and on aërial mycelium of 28 x

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[41] These conidia, though often septate, represent an abbreviated type and thus can be termed microconidia. These microconidia are often referred to as arthrosporial because of their resemblance to the conidia of the genus Arthrosporium.

4.3μ) to sickle-shaped, 5-septate type dominant, in sporodochia and pseudopionnotes, measuring 48.5 x 3.63 (41–61 x 2.9–4.4)μ; in pseudopionnotes, 6- to 9-septate conidia are common, 60–100 x 4.7–5.2μ, from slightly curved to straight and anguiform, apically pointed, distinctly but not prominently pedicellate, in mass typically of light pink-cinnamon color; chlamydospores absent; sporodochia when present often of a large size (up to 3.7 centimeters in diameter); aërial mycelium typically well developed, of uniformly medium fineness, white; substratum, on potato agar rich in glucose, ranging from onion-skin pink and clay color when young to Saccardo's amber in old cultures.

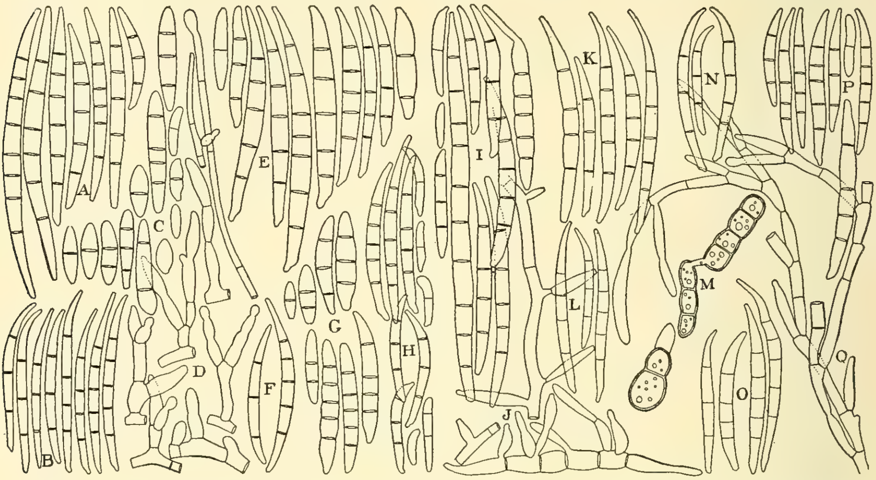

Fig. 16.—*Fusarium diversisporum.* A, *Pseudopionnotal conidia from 26-days-old culture on hard oat agar;* B, *sporodochial conidia,* C, *arthrosporial conidia,* D, *aërial conidiophores producing arthrosporial conidia, from 76-days-old culture on red raspberry cane plug;* E, *pseudopionnotal conidia from 8-days-old culture on hard lima-bean agar with 2 per cent glucose;* F, *typical sporodochial conidia from 113-days-old culture on potato stem plug;* G, *sickle-shaped, normal conidia,* H, *arthrosporial, more or less abnormal, conidia, from aërial mycelium of 45-days-old culture on whole steamed potato tuber;* I, *pseudopionnotal conidia,* J, *mycelial conidiophores and conidiophores produced directly on spores,* K, *conidia, from 10-days-old culture on medium potato agar;* L, *typical sporodochial conidia from 46-days-old culture on whole steamed potato tuber;* M, *chlamydospore-like structures in old conidia from 173-days-old culture on corn agar;* N, *sporodochial conidia from 42-days-old culture on rye straw;* O, *sporodochial conidia from 116-days-old culture on rye straw;* P, *sporodochial conidia from 24-days-old culture on potato tuber plug;* Q, *conidiophore from 24-days-old culture on potato tuber plug*

Hab. On rotted tubers of *Solanum tuberosum,* New York State, in close association with *F. metacroum.*

Latin description.—Conidiis ex arthrosporialibus (brevibus, falciformibus, atque, si 3–septata et in aerio mycelio, plerumque 28 x 4.3μ), falciformibus plerumque 5–septatis, 48.5 x 3.63 (41–61 x 2.9–4.4)μ in sporodochiis pseudopionnotibusque; in pseudopionnotibus, conidiis 6–9–septatis frequentibus, 60–100 x 4.7–5.2μ, parum curvatis rectis, demum anguiformibus, apice acutis, distincte sed non insignite pedicellatis, in totum typice pallide "pinkish cinnamon" (R); nullis chlamydosporis; sporodochiis, si exstant, saepe magnis (usque ad 3.7 cm. diam.); aerio mycelio typice plene maturo, uniformi mediocri subtilitate, albo; substrato—in Solani tuberosi agare perglucoso—in culturis juvenibus, "onion-skin pink" (R) vel "clay-color" (R), in culturis maturis "Saccardo's amber" (R).

Hab. In tuberibus putridis Solani tuberosi una cum F. metacroo, New York, Amer. bor.

Measurements of conidia from cultures on different media are as follows:

On slightly acidified hard potato agar, culture ten days old; conidia from thin pseudopionnotes:

Conidia: 0–septate, rare
1–septate, 7 per cent, 10 x 2.4μ
2–septate, 1 per cent, 20 x 2.9μ
3–septate, 36 per cent, 36 x 3.1 (29–46 x 2.9–3.5)μ
4–septate, 20 per cent, 49 x 3.15 (40–53 x 3–4)μ
5–septate, 35 per cent, 60 x 3.8 (50–70 x 3.5–4.2)μ
6– to 9–septate, 1 per cent (60–100 x 4–5μ)

On red raspberry cane plug, culture seventy-six days old; conidia from a small sporodochium:

Conidia: 3–septate, 57 per cent, 40 x 2.7(32–45 x 2.4–3)μ
4–septate, 28 per cent, 45 x 2.9(42–52 x 2.6–3.2)μ
5–septate, 15 per cent, 50 x 2.9(43–54 x 2.7–3.2)μ

On hard lima-bean agar, culture eight days old; conidia from a thin layer near substratum:

Conidia: 1–septate, 2 per cent
2–septate, 1 per cent
3–septate, 32 per cent, 37 x 3.9 (28–43 x 3.5–5.3)μ
4–septate, 9 per cent

5–septate, 55 per cent, 54 x 4.4 (40–61 x 3.5–5.5)μ
6–septate 61 x 4.9 (60–70 x 4.7–5.2)μ (only four measured)
7–septate 1 per cent 67 x 5 (60–79 x 4.7–5.2)μ (only four measured)
8–septate 76 x 4.8 (70–79 x 4.7–4.8)μ (only three measured)

On potato tuber plug, culture twenty-four days old; conidia from a sporodochium, many spores degenerated, only normal ones measured:

Conidia: 0–septate, none
1–septate, 4 per cent
2–septate, none
3–septate, 50 per cent, 32 x 3.1 (20–40 x 2.7–3.5)μ
4–septate, 22 per cent
5–septate, 24 per cent, 42 x 3.5 (35–51 x 3–3.6)μ

On potato stem plug, culture one hundred and thirteen days old; conidia from a sporodochium:

Conidia: 3–septate, 25 per cent, about 38 x 3.3μ
4–septate, 25 per cent, about 40 x 3.4μ
5–septate, 50 per cent, 42 x 3.5 (36–46 x 3–4.1)μ

On whole steamed potato tuber, culture forty-five days old:

(1) Conidia from aërial mycelium

Conidia: A — Arthrosporial type
1–septate, 10 per cent, about 10 x 3.9μ
2–septate, 3 per cent, 16 x 4μ
3–septate, 71 per cent, 28 x 4.3 (19–37 x 4–5)μ
4–septate, 6 per cent
5–septate, 10 per cent, 41 x 4.2 (37–46 x 4.1–4.5)μ

B — Sickle-shaped type
0–septate, 6 per cent, about 10 x 2.6μ
1–septate, 22 per cent, 16 x 2.8 (13–19 x 2.4–3)μ
2–septate, 4 per cent, about 21 x 2.9μ
3–septate, 40 per cent, 30 x 3.1 (20–34 x 2.9–3.5)μ
4–septate, 12 per cent, about 39 x 3.2μ
5–septate, 16 per cent, 45 x 3.3 (38–53 x 3–3.6)μ

(2) Conidia from small sporodochia converging into a nearly continuous layer

Conidia: 3–septate, 45 per cent, 33 x 3.1 (20–41 x 2.5–3.5)μ
4–septate, 28 per cent, 40 x 3.2μ (only a few measured)
5–septate, 27 per cent, 43 x 3.3 (36–50 x 2.9–4.1)μ

On hard lima-bean agar, culture thirteen days old; conidia from pseudopionnotal stage:

Conidia: 3–septate, about 12 per cent, 48 x 3μ (only three measured)
4–septate, about 8 per cent
5–septate, 80 per cent, 61 x 3.3 (50–70 x 3.1–3.5)μ

On hard oat agar, culture thirty-seven days old; conidia from aërial mycelium close to substratum:

Conidia: 1–septate, 1 per cent
3–septate, 20 per cent, 31 x 3.3μ
4–septate, 15 per cent
5–septate, 60 per cent, 45 x 3.7 (43–49 x 3.3–4)μ
6–septate, 4 per cent, 61 x 4.2μ (only one measured)

On same medium as above, culture twenty-four days old; conidia from pseudopionnotes:

Conidia: 3–septate, 10 per cent, 28 x 3.3μ
4–septate, 4 per cent
5–septate, 50 per cent, 51 x 4μ
6–septate, 30 per cent, 60.6 x 4.2μ
7– and 8–septate, 6 per cent, 75 x 43μ

Average of the above measurements:

Conidia: 0–septate, about 0.5 per cent, 10 x 3.25μ
1–septate, about 4 per cent, 12 x 3.0μ
2–septate, about 0.5 per cent
3–septate, about 35.5 per cent, 36 x 3.2μ
4–septate, about 15 per cent
5–septate, about 44 per cent, 48.5 x 3.63μ
6– to 9–septate, about 0.5 per cent, 60–100 x 4.7–5.2μ

The most characteristic features of the fungus are as follows: 0– to 3–septate, spindle-shaped conidia of aërial mycelium; 3– to 5–septate, very narrow sickle-shaped conidia of sporodochia, and comparatively broad and longer anguiform; 6– to 9–septate conidia of pseudopionnotal stage often occurring on various agars.

20. **Fusarium biforme** n. sp. (Figs. 1U and 17; Pl. VII, fig. 10)

Conidia of two forms: sporodochial conidia more or less uniformly ellipsoid, 3– to 5–septate, 3–septate measuring 39 x 3.3 (36–46 x 2.9–3.6)μ, 5–septate measuring 51 x 3.5 (43–60 x 3–4)μ; pseudopionnotal conidia 0– to 9– and even up to 12–septate, sometimes nearly straight or anguiform, 5–septate measuring 52 x 4.2μ, 6– to 9–septate measuring 45–84 x 4–5.8μ; conidia in mass typically salmon-colored; no chlamydospores; mycelium well developed, uniform, white in pseudopionnotal stage and from pink to pomegranate near substratum in sporodochial stage (on hard oat agar);[42] substratum from brick red to pomegranate (on oat and other agars).

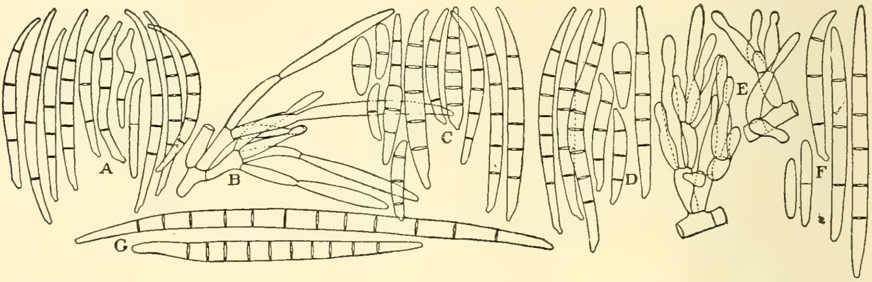

FIG. 17.—*Fusarium biforme.* A, *Conidia (sporodochium 3 millimeters in diameter),* B, *conidiophore, from 29-days-old culture on hard oat agar;* C, *conidia from aërial mycelium from 71-days-old culture on potato tuber plug;* D, *semi-pseudopionnotal conidia from 14-days-old culture on slightly acidified hard potato agar;* E, *conidiophores,* F, *typical conidia,* G, *largest and most highly septate conidia, from 10-days-old culture on rice*

Hab. On rotted tuber of *Solanum tuberosum,* together with *F. coeruleum,* Wisconsin.

Differs from *F. diversisporum* chiefly in color of mycelium and of substratum on hard oat agar and other agars, and in absence of a uniform and typical arthrosporial stage of aërial conidia, although single conidia of that type occur here also. Differs from *F. diffusum* by the presence of 8– to 12–septate conidia.

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[42] On the same medium *F. diversisporum* remains from white to clay in color.

Latin description.—Conidiis biformibus: conidiis sporodochialibus, plus minusve aequabiliter ellipsoidalibus, 3–5–septatis: 3–septatis, 39 x 3.3 (36–46 x 2.9–3.6)μ; 5–septatis 51 x 3.5 (43–60 x 3–4)μ; conidiis pseudopionnotalibus 0–9–septatis vel etiam 12–septatis, interdum prope rectis vel anguiformibus; 5–septatis, 52 x 4.2μ; 6–9–septatis, 45–84 x 4–5.8μ; conidiis in totum typice "salmon-color" (R); nullis chlamydosporis; mycelio plene maturo, uniformi, albo in pseudopionnotum gradu, ex rubello "pomegranate" (R) in gradu sporodochiali (in durae avenae agare); substrato e "brick red" (R) "pomegranate" (R) (in avenae agaribus et aliis agaribus).

Hab. In tuberibus putridis Solani tuberosi una cum F. coeruleo, Wisconsin, Amer. bor.

Measurements of the conidia on different media are as follows:

On corn agar, culture twenty-six days old; conidia from a thin layer directly on substratum;

Conidia: 1– to 4–septate, 20 per cent (about an equal number of each)
5–septate, 45 per cent, 60 x 3.6 (50–70 x 3.4–3.8)μ
6– to 9–septate, 35 per cent, 80 x 4.4 (77–88 x 4.1–4.7)μ

On hard potato agar, slightly acidified, culture fourteen days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 0.5 per cent, 12 x 3μ
1–septate, 3 per cent, 19 x 3μ
2–septate, rare, 25 x 3.2μ
3–septate, 5 per cent, 32 x 3.7 (24–51 x 3.5–4.2)μ
4–septate, 2.5 per cent
5–septate, 80 per cent, 51.5 x 4.3 (40–74 x 4–5.2)μ
6–septate, 6 per cent, 58 x 4.7 (50–75 x 4.3–5.3)μ
7– and 8–septate, 3 per cent, 68 x 4.7 (55–80 x 4.3–5.3)μ
9– to 11–septate, rare, 84 x 5.9μ (only a few measured)

On potato tuber plug, culture seventy-one days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 2 per cent, 9 x 3μ
1–septate, 6 per cent, 14 x 3.3μ, a few 15 x 5.7μ
2–septate, 3 per cent, 20 x 3.4μ
3–septate, 30 per cent, 27.5 x 3.9 (19–39 x 3.5–4.1)μ
4–septate, 12 per cent

5–septate, 44 per cent, 43 x 4.1 (31-54 x 3.9–5.2)μ
6–septate, 2 per cent, 49 x 4.3 (45–72 x 4–4.9)μ
7–septate, 1 per cent, 60 x 4.8 (59–73 x 4.1–5.3)μ
8– and 9–septate, rare, 60–78 x 4.5–5.8μ (only a few measured)

On hard lima-bean agar with 2 per cent glucose, culture seven days old; conidia from aërial mycelium:

Conidia: 0– and 1–septate, 79 per cent
3–septate, 15 per cent, 36 x 4.6 (33–41 x 4.3–4.7)μ
4–septate, 5 per cent
5–septate, 1 per cent, 54 x 4.7 (53–66 x 4.3–5.3)μ

On hard oat agar, culture twenty-eight days old; conidia from a large (3 millimeters in diameter) sporodochium:

Conidia: 1–septate, 3 per cent, about 28 x 3 (28–37 x 2.3–3)μ
2–septate, 1 per cent
3–septate, 45 per cent, 39 x 3.3 (36–46 x 2.9–3.6)μ
4–septate, 16 per cent
5–septate, 35 per cent, 51 x 3.5 (43–60 x 3–4)μ

Average of the above measurements for all non-sporodochial conidia:

Conidia: 0–septate, about 9 per cent, 11 x 3μ
1–septate, about 11 per cent, 16.5 x 3.15μ
2–septate, about 2 per cent
3–septate, about 20 per cent, 32 x 4.1μ
4–septate, about 8 per cent
5–septate, about 41 per cent, 52 x 4.2μ
6– to 9–septate, about 9 per cent, 45–84 x 4–5.8μ

The organism when first isolated had large sporodochia (from 2 to 5 millimeters in diameter), and the substratum and mycelium near it were from rose to pomegranate in color; later the color of mycelium and substratum faded and no sporodochia were produced at all; during the last year its original characters — both color and sporodochia — reappeared. Under just what influence loss of characters and their reappearance took place the writer is unable to say, although it seems that a slightly acidified and relatively dry medium actually helped to bring the fungus to the original conditions.

21. **Fusarium anguioides** n. sp. (Figs. 1T and 18; Pl. VI, fig. 11)

Conidia of diverse type, ranging from arthrosporial (short spindle-shaped, with more or less rounded ends, 0– to 3–septate) to typically slightly curved or nearly straight and anguiform, 1– to 15–septate; 1– and 3–septate conidia typical for the first form and measuring 27 x 4.4 (20–38 x 3.9–5.3)μ; for the other form the conidia commonly measuring as follows:

5–septate, 51 x 4.2 (47–68 x 3.9–4.6)μ
6– and 7–septate, 76 x 4.6 (65–86 x 4.2–5.2)μ
8– and 9–septate, 89 x 4.86 (80–102 x 4.3–5.8)μ

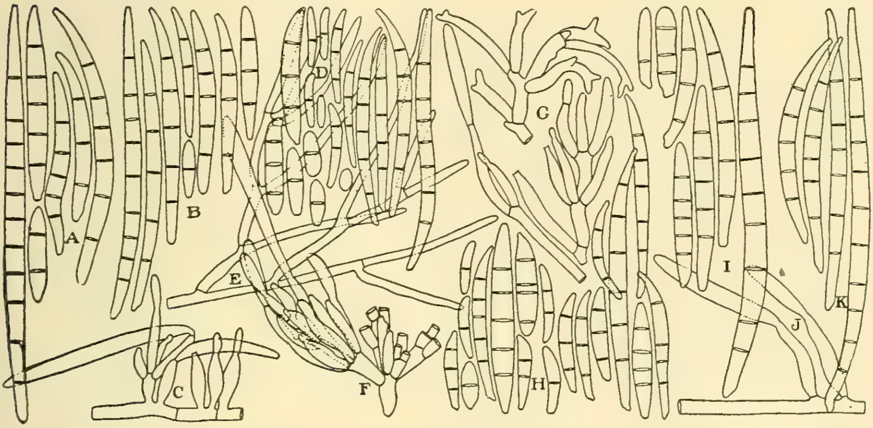

FIG. 18.—*Fusarium anguioides.* A, *Pseudopionnotal conidia from 6-days-old culture on slightly acidified hard potato agar;* B, *conidia from 15-days-old culture on wheat grain;* C, *conidiophores from 6-days-old culture on slightly acidified hard potato agar;* D, *conidia from 46-days-old culture on wheat grain;* E, *conidiophore from 15-days-old culture on wheat grain;* F *and* G, *conidiophores,* H, *conidia, from 62-days-old culture on red raspberry cane plug;* I, *pseudopionnotal conidia from 11-days-old culture on medium potato agar;* J, *conidiophores from 62-days-old culture on red raspberry cane plug;* K, *pseudopionnotal conidia from 13-days-old hard lima-bean agar*

Color of conidia in pseudopionnotal layer, on glucose potato agar, ranging from light pinkish cinnamon to cinnamon; arthrosporial conidia of common occurrence on aërial mycelium, but often the latter, especially on different agar, nearly absent, when a thin spore layer, pseudopionnotes, is produced for which anguiform conidia are typical.

Hab. On rotted tuber of *Solanum tuberosum* from Castile, New York, in association with *F. arcuosporum.*

In pseudopionnotal stage *F. anguioides* is much like *F. biforme,* but has no sporodochial stage, or rather no macroscopically observable sporodochia.

Latin description.—Conidiis variis typis, interdum arthrosporialibus (brevibus, fusiformibus, terminis plus minusve rotundatis, 0–3–septatis), interdum typice paulum curvatis vel prope rectis anguiformibusque, 1–15–septatis; conidiis primo typo typice 1–vel 3–septatis, 27 x 4.4 (20–38 x 3.9–5.3)μ; conidiis altero typo: 5–septatis plerumque 51 x 4.2 (47–68 x 3.9–4.6)μ; 6–7–septatis plerumque 76 x 4.6 (65–86 x 4.2–5.2)μ; 8–9–septatis plerumque 89 x 4.86 (80–102 x 4.3–5.8)μ. Conidiis in strato pseudopionnotali, in agari glucoso Solani tuberosi e pallide "pinkish-cinnamon" (R) "cinnamon" (R); saepe conidiis arthrosporialibus in aerio mycelio, sed hoc mycelio saepe — imprimis in alio agari — prope absente, quae cum ita sint tenues sporarum strati, pseudopionnotes, oriuntur cum conidiis typice anguiformibus.

Hab. In tuberibus putridis Solani tuberosi, una cum F. arcuosporo, Castile, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On red raspberry cane plug, culture sixty-two days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 3 per cent
1–septate, 19 per cent, 17 x 3 (12–22 x 2.5–4)μ
2–septate, 9 per cent
3–septate, 43 per cent, 30 x 3.5 (17–42 x 3–4.8)μ
4–septate, 7 per cent
5–septate, 18 per cent, 47 x 4.2 (35–55 x 3–5.2)μ, the thickest 50 x 5.9μ
6–septate, 1 per cent
7–septate, rare, the longest 75 x 4.8μ

On hard potato agar, culture eleven days old; conidia from thin pseudopionnotes:

Conidia: 1–septate, 3 per cent, 18 x 4.3μ (only a few measured)
2–septate, 2 per cent
3–septate, 20 per cent, 29 x 4.5 (20–35 x 4–6.5)μ, the broadest 34 x 6.5μ
4–septate, 10 per cent

5–septate, 44 per cent, 52 x 4.6 (33–75 x 4.1–6.2)μ

6–septate 7–septate	9 per cent	72 x 5.2μ (only a few measured)
8–septate 9–septate	8 per cent	80 x 5.3μ (only a few measured) 102 x 5.8μ (only a few measured, the largest 105 x 6.5μ)
11–septate 12–septate	4 per cent	91 x 5.8μ (only one measured) 101 x 5.7μ (only one measured)

On hard lima-bean agar, culture thirteen days old; conidia from thin pseudopionnotes:

Conidia: 5–septate, 9 per cent, 58 x 4.1 (53–62 x 3.9–4.4)μ
6–septate, 12 per cent, 78 x 4.3 (70–87 x 4.1–4.5)μ
7–septate, 19 per cent, 86 x 4.3 (78–96 x 4.1–4.5)μ
8–septate, 32 per cent, 93 x 4.4 (86–100 x 4.2–4.8)μ
9–septate, 17 per cent, 94 x 4.4 (90–100 x 4.2–4.7)μ
10–septate, 7 per cent, 94 x 4.4 (97–103 x 4.3–4.5)μ (only four measured)
11–septate, 2 per cent, 108 x 4.4μ (only one measured)
12–septate, 1 per cent, 110 x 4.4μ (only one measured)
13–septate, 1 per cent, 126 x 4.8μ (only one measured)

On wheat grain, culture fifteen days old; conidia from aërial mycelium:

Conidia: 0–septate, very rare
1–septate, very rare
2–septate, very rare, 26 x 3.1μ (only a few measured)
3–septate, 5 per cent
4–septate, 2.5 per cent
5–septate, 89 per cent, 60 x 4.2 (45–70 x 3.9–4.7)μ

6–septate 7–septate	3 per cent	75 x 4.2 (67–80 x 4.1–4.4)μ (only a few measured) 85 x 4.3μ (only a few measured)
8–septate 9–septate	0.5 per cent	81 x 4.6μ (only a few measured) 86 x 4.5μ (only a few measured, the longest 92 x 4.5μ)

On hard lima-bean agar, culture nine days old; conidia from pseudo pionnotes:

Conidia: 0–septate
1–septate } 1 per cent
2–septate
3–septate, 2 per cent, 45 x 4 (only a few measured)
5–septate, 40 per cent, 63 x 4.3 (43–76 x 3.5–4.7)μ
6–septate, 22 per cent, 72 x 4.4 (62–79 x 4–4.7)μ
7–septate, 18 per cent, 80 x 4.5 (63–88 x 4.3–4.8)μ
8–septate, 10 per cent, 87 x 4.7 (75–93 x 4.3–4.8)μ
9–septate, 5 per cent, 88 x 4.7μ (only two measured)
10–septate, 2 per cent, 90 x 4.8μ (only one measured)

On wheat grain, culture forty-six days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 3 per cent: arthrosporial, 15 x 4.1 (10–22 x 3.9–4.4)μ; sickle-shaped, 20 x 2.8 (16–22 x 2.6–3.5)μ
1–septate, 27 per cent
2–septate, 4 per cent } arthrosporial, 27 x 4.4 (20–38 x 3.9–5.3)μ
3–septate, 38 per cent
4–septate, 11 per cent
5–septate, 15 per cent, 53 x 3.9 (42–62 x 3.5–4.2)μ
6–septate, 2 per cent, 65 x 4.2 (54–70 x 3.9–5.2)μ
7– and 8–septate, rare, 73–81 x 4.1–4.8μ (only three measured)

Average of the above measurements:

Conidia: 0–septate, 0.6 per cent
1–septate, 4.6 per cent
2–septate, 2.2 per cent
3–septate, 14 per cent
4–septate, 4 per cent
5–septate, 40 per cent, 50 x 4.2μ
6– and 7–septate, 16.8 per cent, 76 x 4.6μ
8– and 9–septate, 14.4 per cent, 89 x 4.86μ
10– to 15–septate, 3.4 per cent, 103 x 4.9μ (the largest conidium found was 15–septate, 150 x 6μ)

22. *Fusarium anguioides* var. **caudatum** n. var. (Fig. 19; Pl. vi, fig. 9)

Conidial type very much the same as that of *F. anguioides*, but 8- to 11-septate conidia very rare and the size for the same septation somewhat larger; conidia never in macroscopically observable thick pseudopionnotes as is often the case in *F. anguioides* when grown on various agars. Conidia of this fungus, even in comparatively young cultures, often produce on one end a long, usually unbranched, germ tube (see certain conidia in figure 19), which has never been observed in *F. anguioides*.

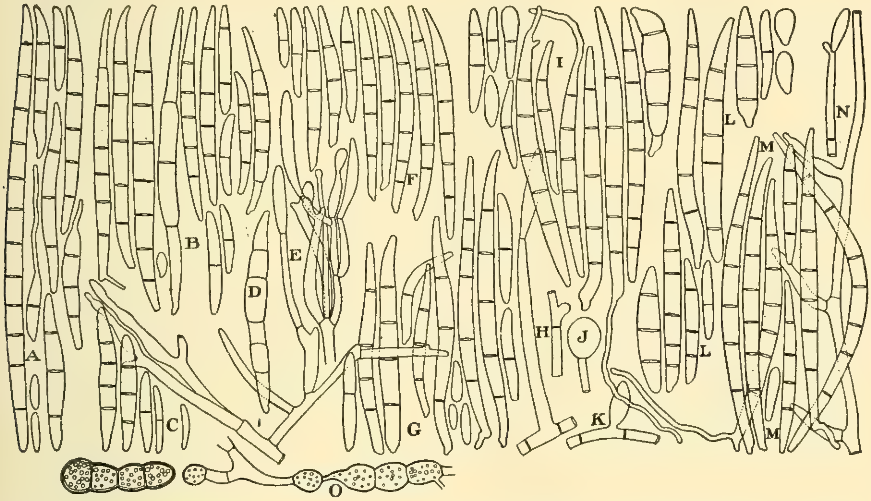

FIG. 19.—*Fusarium anguioides var. caudatum.* A, *Pseudopionnotal conidia from 10-days-old culture on slightly acidified hard potato agar;* B, *conidia from 61-days-old culture on red raspberry cane plug;* C, *typical,* D, *degenerated, conidia,* E, *conidiophores, from 67-days-old culture on potato stem plug;* F, *conidia from 15-days-old culture on wheat grain;* G, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar;* H, *conidiophores,* I, *pseudopionnotal conidia,* J, *chlamydospore-like mycelial swelling,* K, *conidiophores, from 11-days-old culture on medium potato agar;* L, *pseudopionnotal conidia from 16-days-old culture on hard lima-bean agar with 2 per cent glucose;* M, *pseudopionnotal conidia from 37-days-old culture on hard oat agar;* N, *conidiophores from 11-days-old culture on medium potato agar*

Hab. On rotted tubers of *Solanum tuberosum*, in association with *F. coeruleum*, Ithaca, New York.

Measurements of conidia from cultures on different media are as follows:

On red raspberry cane plug, culture sixty-one days old:

Conidia: 0– and 1–septate, 10 per cent
2– to 4–septate, 35 per cent
5–septate, 55 per cent, 56 x 4.2 (35–80 x 3.7–4.8)μ
6–septate, rare, the longest 85 x 5.2μ

On hard lima-bean agar, culture sixteen days old:

Conidia: 0–septate, 17 per cent, 13 x 2.7 (11–16 x 2.3–3.7)μ
1–septate, 18 per cent, 22 x 3.3 (18–28 x 3–4.4)μ, the broadest 33 x 6.1μ
2–septate, 12 per cent
3–septate, 40 per cent, 38.5 x 4 (29–45 x 3.5–4.7)μ, the broadest 33 x 6.1μ
4–septate, 3 per cent
5–septate, 7 per cent, 60 x 4.5 (52–75 x 4.1–5.4)μ
6–septate, 3 per cent, 67 x 5.2 (53–77 x 4.1–6.1)μ
7–septate, very few, 82 x 5.2μ (only one measured)
8–septate, few, 75 x 5.3 (72–88 x 5–5.6)μ (only two measured)
9–septate, very few, 94 x 5.2μ (only one measured)

On hard potato agar, culture eleven days old:

Conidia: 0–septate, 12 per cent, 14 x 3.9 (8.7–20 x 2.6–4.2)μ
1–septate, 11 per cent, 17 x 4.1μ (only four measured)
2–septate, 9 per cent
3–septate, 47 per cent, 40 x 4.3 (25–53 x 3.5–5.3)μ
4–septate, 8 per cent
5–septate, 12 per cent, 66 x 4.6 (50–77 x 4–6.1)μ

$$\left.\begin{array}{l}\text{6–septate,}\\ \text{7–septate,}\end{array}\right\}\text{ 1 per cent }\left\{\begin{array}{l}\text{79 x 5.3}\mu\text{ (only one measured)}\\ \text{85 x 5.2}\mu\text{ (only one measured)}\end{array}\right.$$

On wheat grain, culture fifteen days old:

Conidia: 0–septate, 4 per cent
1–septate, 40 per cent, 24 x 3.1 (15–27 x 2.7–3.6)μ
2–septate, 10 per cent
3–septate, 42 per cent, 43 x 3.8 (36–62 x 3.5–5.2)μ
4–septate, 2 per cent
5–septate, 2 per cent, 55 x 4.1 (43–62 x 4–4 5)μ
6–septate, few, 57 x 4.3μ (only one measured)
7–septate, very few, 79 x 4.3μ (only one measured)

On hard lima-bean agar, culture nine days old:

Conidia: 0–septate, 14 per cent
1–septate, 28 per cent
2–septate, 5 per cent
3–septate, 32 per cent, 44 x 3.6 (29–63 x 3–4)μ (exceptionally 4.8μ in diameter)
4–septate, 5 per cent
5–septate, 16 per cent, 65 x 3.9 (52–79 x 3.5–4.8)μ

Average of the above measurements:

Conidia: 0–septate, about 10.4 per cent, 13.5 x 3.3μ
1–septate, about 20.4 per cent, 21 x 3.5μ
2–septate, about 9.2 per cent
3–septate, about 35.2 per cent, 41 x 3.9μ
4–septate, about 5.6 per cent
5–septate, about 18.4 per cent, 60.4 x 4.26μ
6– and 7–septate, about 0.8 per cent, 82 x 4.9μ
8– and 9–septate, very rare, 85 x 5.2μ

23. **Fusarium arthrosporioides** n. sp. (Figs. 1, L to P, and 20; Pl. III, figs. 3 and 4; Pl. VII, fig. 11)

Conidia of three types: (1) elliptical, apically attenuate, slightly pedicellate, mostly 5–septate, 48 x 4.3 (45–55 x 4–4.7)μ; (2) arthrosporial, 0– to 3–(5–)septate, broad spindle-shaped, 3-septate measuring about 25 x 5.4 (19–33 x 4.7–6)μ; (3) sporotrichial, mostly 0–septate, 4.5 x 3.8 (2.9–9 x 2.6–8)μ. Type 1 is found mostly in pseudopionnotes, type 2 on aërial mycelium, and type 3 mainly in small, dense, bushlike clusters of conidiophores, often resembling sporodochia; no true chlamydospores; aërial mycelium from white to light tints of rose and salmon hues, uniform or covered with knotted, more or less large, areas; substratum from clay color to buckthorn brown and bright red (Plate III, fig. 4). The organism occupies an intermediate position between sections Arthrosporiella and Sporotrichiella.

Hab. In discolored tissues of tuber of *Solanum tuberosum*, Ireland.

The organism remained for a long time in pure culture, with well-developed aërial mycelium and with a bright red color (Plate III, fig. 4); the dominant type of conidia was sickle-shaped, 5–septate. During the last year — the second year of its cultivation — the development of aërial mycelium has become poorer, the color of the substratum has changed to clay color and buckthorn brown, and sporotrichic conidia are the commonest type. Whether the organism has changed only temporarily or not is as yet unknown.[43]

Latin description.—Conidiis tribus typis: (1) conidiis ellipticis, apice attenuatis, paulum pedicellatis, plerumque 5-septatis, 48 x 4.3 (45–55 x 4–4.7)μ; (2) conidiis arthrosporialibus, 0–3(5)-septatis, latis fusiformibus, 3-septatis circa 25 x 5.4 (19–33 x 4.7–6)μ; (3) conidiis sporotrichialibus, plerumque 0-septatis, 4.5 x 3.8 (2.9–9 x 2.6–8)μ. Typo primo plerumque in pseudopionnotibus, typo altero in aerio mycelio, typo tertio plerumque in parvis densis fruticosis conidiophoris uveosis, saepe similibus sporodochiis; nullis veris chlamydosporis; aerio mycelio ex albo pallide roseo "salmon color" (R), uniformi vel nodosas plus minusve magnas areas

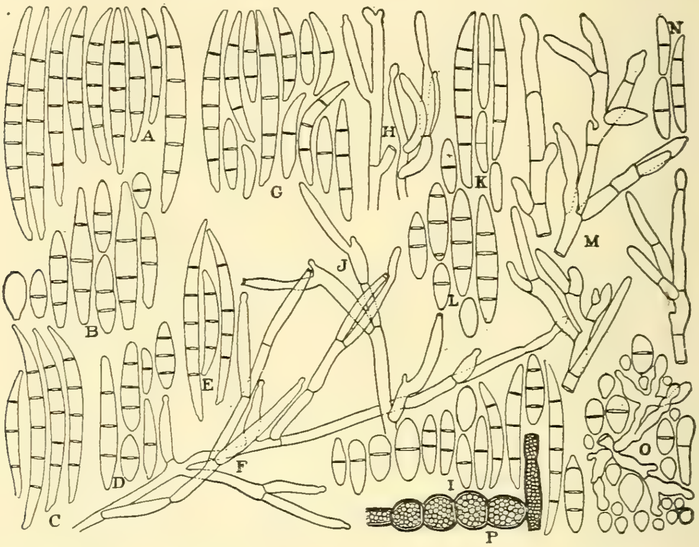

FIG. 20.—*Fusarium arthrosporioides.* A, *Normal pseudopionnotal conidia,* B, *arthrosporial conidia, from 14-days-old culture on hard lima-bean agar;* C, *sporodochial conidia from 64-days-old culture on hard lima-bean agar with 2 per cent glucose;* D, *arthrosporial,* E, *normal, conidia,* F, *conidiophores, from 11-days-old culture on medium potato agar;* G, *aërial conidia,* H, *conidiophores, from 11-days-old culture on slightly acidified hard potato agar;* I, *conidia and chlamydospores from 76-days-old culture on red raspberry cane plug;* J, *conidiophores from 11-days-old culture on medium potato agar;* K, *normal,* L, *arthrosporial, type of conidia,* M, *conidiophores, from aërial mycelium of 16-days-old culture on hard lima-bean agar with 2 per cent glucose;* N, *sickle-shaped conidia,* O, *arthrosporial and sporotrichial conidia and conidiophore of the sporodochial stage, from 36-days-old culture on rye grain;* P, *chlamydospores*

exhibente; substrato ex argillaceo "buckthorn brown" (R) vel nitide rubro (Tab. III, fig. 4). Fungo locum medium inter Sectiones Arthrosporiellam et Sporotrichiellam obtinente.

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[43] In this connection see last paragraph in description of *F. biforme* (page 168).

Hab. In textibus decoloratis Solani tuberosi tuberum, Hibernia.

Measurements on different media are as follows:

On slightly acidified hard potato agar, culture eleven days old; conidia from aërial mycelium:

Conidia: 0–septate, 0.5 per cent, 12 x 2.7μ
1–septate, 15.5 per cent, 25 x 3.8 (17–30 x 2.6–4.7)μ
2–septate, 3 per cent, 27 x 4μ
3–septate, 77.5 per cent, 35 x 3.5 (22–48 x 3–4.4)μ
4–septate, 3 per cent, 43 x 4.2 (35–50 x 3.5–4.8)μ
5–septate, 0.5 per cent, 47 x 4.4μ (only a few measured)

On hard lima-bean agar with 2 per cent glucose, culture sixteen days old:

(1) Sickle-shaped conidia
0–septate, 4.7 per cent, 10 x 2.6μ (only a few measured)
1–septate, 4.7 per cent, 19 x 3.1 (14–24 x 2.9–3.5)μ
2–septate, few, 28 x 3.3μ (only a few measured)
3–septate, 5.9 per cent, 34 x 4 (31–40 x 3.3–4.7)μ
4–septate, 0.5 per cent, 43 x 4.3 (35–44 x 4.1–4.7)μ
5–septate, 0.6 per cent, 47 x 4.4 (43–54 x 4.1–4.8)μ

(2) Arthrosporial conidia
0–septate, 13 per cent, 8.5 x 4.3 (6–12 x 3.5–6.4)μ
1–septate, 23.5 per cent, 15 x 4.4 (9–20 x 3.5–5.3)μ
2–septate, 11.7 per cent, 18 x 5.2 (13–35 x 4.7–6)μ
3–septate, 35.4 per cent, 25 x 5.4 (19–33 x 4.7–6)μ
4–septate, few, 39 x 6 (33–47 x 5–7)μ
5–septate, few, 46 x 5.7 (42–56 x 5.2–6)μ
The largest conidium observed was 8–septate (exceptionally close-septate), and measured 58 x 6.5μ

On potato stem plug, culture one hundred and seven days old:
Conidia: 1–septate, 52 per cent, about 13 x 3.5μ
2–septate, 7 per cent
3–septate, 31 per cent, 34 x 3.4μ
4–septate, 3 per cent, 43 x 3.6μ
5–septate, 7 per cent, 45 x 4 (40–50 x 3.4–4.3)μ

On hard lima-bean agar, culture sixty-four days old; conidia from a sporodochium:
Conidia: 1–septate, 1 per cent
3–septate, 12 per cent, 26 x 3.4 (22–44 x 3–3.6)μ
4–septate, 5 per cent, 43 x 3.9μ (only two measured)
5–septate, 81 per cent, 48 x 4 (43–52 x 3.5–4.3)μ
6–septate, 1 per cent, about 50 x 4.1μ (only two measured)

On hard lima-bean agar, culture fourteen days old; conidia from a dense mycelial growth at the bottom of the slant:
Conidia: 0–septate, 1.5 per cent, 13 x 4.8μ (only three measured)
1–septate, 10 per cent, 20 x 5.2 (9–27 x 4.1–7)μ
2–septate, 4 per cent, 22 x 8.3μ (only three measured)
3–septate, 38 per cent, 28 x 5.2 (19–39 x 4.3–5.9)μ
4–septate, 4 per cent
5–septate, 37 per cent, 55 x 4.7 (43–70 x 4.4–5.3)μ
6–septate, 4 per cent, 61 x 5 (54–70 x 4.8–5.3)μ
7–septate, 1.5 per cent, 69 x 5 (64–79 x 4.7–5.3)μ

On rye grain, culture thirty-six days old; conidia from a sporodochium, of sporotrichial type:
Conidia: 0–septate, 97 per cent, 4.5 x 3.8 (2.9–9 x 2.6–8)μ
1–septate, 3 per cent, 10.2 x 5.6 (8.5–14 x 4.2–6.3)μ
2–septate, rare
3–septate, rare

Average of the above measurements:
Conidia: 0–septate, 17 per cent $\begin{cases}\text{4.5 x 3.8μ (sporotrichial form)}\\ \text{12.1 x 3.8μ (arthrosporial form)}\end{cases}$
1–septate, 18 per cent, 17 x 14.2μ
2–septate, 6 per cent
3–septate, 33 per cent, 25 x 5.4μ (arthrosporial form)

4–septate, 3 per cent
5–septate, 21 per cent, 48 x 4.3μ (sickle-shaped)
6–septate, 1 per cent, 55.5 x 4.55μ (sickle-shaped)
7–septate, 0.5 per cent, 69 x 5μ (sickle-shaped)

In the case of this organism, the average percentage of septation of conidia is of no value because of the great diversity of the forms occurring. Therefore it is a safer basis to consider each stage (sporodochial and from aërial mycelium) by itself separately.

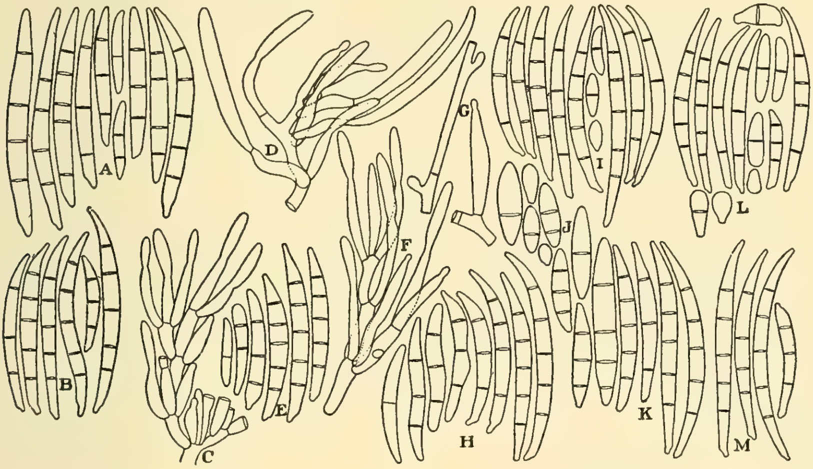

FIG. 21.—*Fusarium arthrosporioides var. asporotrichius.* A, *Aërial conidia from 7-days-old culture on hard lima-bean agar with 2 per cent glucose;* B, *sporodochial conidia from 82-days-old culture on potato tuber plug;* C, D, *conidiophores, from 11-days-old culture on slightly acidified hard potato agar;* E, *aërial conidia from 25-days-old culture on potato tuber plug;* F *and* G, *conidiophores,* H, *pseudopionnotal conidia, from 11-days-old culture on slightly acidified hard potato agar;* I, *conidia from 68-days-old culture on red raspberry cane plug;* J, *arthrosporium-like,* K, *normal, conidia from aërial mycelium from 14-days-old culture on hard lima-bean agar;* L, *aërial conidia from 36-days-old culture on rye grain;* M, *sporodochial conidia from 14-days-old culture on hard lima-bean agar*

24. *Fusarium arthrosporioides* var. **asporotrichius** n. var. (Figs. 1, R and S, and 21; Pl. VII, fig. 9)

This fungus is very much the same as *F. arthrosporioides,* but differs from the latter by typical absence of sporotrichial sporodochia and by more pronounced and common production of from medium to large (up to 3 millimeters in diameter) sporodochia with sickle-shaped, 5–septate conidia, 51.2 x 4.3 (46–59 x 4.15–4.7)μ, as dominant type.

Hab. On rotted tubers of *Solanum tuberosum* in association with *F. Solani* and *F. coeruleum*, New York State.

Measurements on different media are as follows:

On slightly acidified hard potato agar, culture eleven days old, conidia from pseudopionnotes:

Conidia: 0–septate, 0.8 per cent
1–septate, 3.4 per cent, 20 x 3.3μ
2–septate, 2.5 per cent, 30 x 3.8μ
3–septate, 49 per cent, 40 x 4 (30–56 x 3–4.7)μ
4–septate, 21.8 per cent, 50 x 4.3 (43–60 x 3.9–4.5)μ
5–septate, 22.5 per cent, 53.5 x 4.4 (49–62 x 4–4.7)μ

On red raspberry cane plug, culture sixty-eight days old; conidia from a sporodochium:

Conidia: 1–septate, very rare
2–septate, very rare
3–septate, 9 per cent, 41 x 3.7 (21–48 x 3.3–4.4)μ
4–septate, 24 per cent, 46 x 4.1 (36–60 x 3.8–4.7)μ
5–septate, 67 per cent, 51 x 4.2 (42–62 x 3.9–4.7)μ
6–septate, rare, 62–70 x 4.1–4.8μ (only a few measured)

On potato tuber plug, culture eighty-two days old; conidia from a sporodochium:

Conidia: 1–septate, rare
2–septate, rare
3–septate, 6 per cent, 36 x 3.8 (25–42 x 3.5–4.1)μ
4–septate, 17 per cent
5–septate, 77 per cent, 49 x 4.2 (43–55 x 3.5–4.7)μ

On hard lima-bean agar, culture seven days old; conidia from aërial mycelium:

Conidia: 0–septate, 3 per cent
1–septate, 8 per cent
2–septate, 8 per cent
3–septate, 65 per cent, 39 x 4.1 (31–67 x 3.9–5.7)μ, the longest 68 x 4.4μ
4–septate, 14 per cent, 49 x 4.7 (44–60 x 4.2–5.3)μ
5–septate, 2 per cent, 59 x 4.8 (52–63 x 4.3–5.7)μ

On potato tuber plug, culture twenty-five days old; conidia from aërial mycelium:

Conidia: 0–septate, rare, about 16 x 2.5μ
1–septate, 7 per cent, 22 x 3.7μ (only three measured)
2–septate, 6 per cent, 27 x 4.1μ (only three measured)
3–septate, 40 per cent, 35 x 4.3 (29–45 x 3.8–4.8)μ
4–septate, 28 per cent, 43 x 4.7 (35 x 49 x 4.1–5.2)μ, the broadest 51 x 5.9μ
5–septate, 19 per cent, 45 x 4.7 (40–53 x 4.3–4.9)μ
6–septate, only one observed, 78 x 5.2μ

On whole steamed potato tuber, culture forty-six days old:

(1) Conidia from a medium large (2 millimeters in diameter) sporodochium

Conidia: 1–septate, 2.5 per cent, about 16 x 3μ
2–septate, 2.5 per cent, about 28 x 3.5 (20–40 x 3.3–3.8)μ
3–septate, 31 per cent, 38.5 x 3.7 (19–44 x 3–4.1)μ
4–septate, 34 per cent, 45 x 4μ (only three measured)
5–septate, 30 per cent, 48 x 4.15 (44–53 x 3.9–4.7)μ
6–septate, very rare, 61 x 4.3μ (only one measured)

(2) Conidia from a small sporodochium

Conidia: 1–septate, 2 per cent
2–septate, rare
3–septate, 51 per cent, 40 x 3.9 (35–43 x 3–4.1)μ
4–septate, 24 per cent, 42 x 4.1 (36–50 x 3.5–4.8)μ
5–septate, 23 per cent, 46 x 4.2 (42–50 x 4–4.5)μ

On medium potato agar, culture eleven days old; conidia from pseudopionnotes:

Conidia: 3–septate, 13 per cent, 39 x 4.3 (28–48 x 3.5–4.8)μ
4–septate, 8 per cent
5–septate, 79 per cent, 48 x 4.6 (41–61 x 3.8–4.9)μ

On hard lima-bean agar, culture fourteen days old:

(1) Conidia from aërial mycelium

Conidia: 0–septate, 6 per cent, 11 x 4.6 (6–18 x 4–5.3)μ
1–septate, 13 per cent, 18 x 5 (10–25 x 4.1–5.5)μ

2–septate, 10 per cent, 24 x 5.1 (20–30 x 4.3–5.5)μ (only three measured)
3–septate, 39 per cent, 31 x 5.2 (22–43 x 4.1–6.2)μ
4–septate, 7 per cent
5–septate, 25 per cent, 56 x 4.7 (42–70 x 4–5.9)μ
6–septate, very few, 63 x 4.9μ (only one measured)

(2) Conidia from a sporodochium
Conidia: 3–septate, about 5 per cent, 36 x 3.1μ (only a few measured)
4–septate, about 5 per cent
5–septate, about 90 per cent, 58 x 4 (52–65 x 3.5–4.8)μ

On rye grain, culture thirty-six days old:
Conidia: 0–septate, 3 per cent
1–septate, 6 per cent
2–septate, 2 per cent
3–septate, 55 per cent, 38 x 3.4 (27–50 x 3–4.4)μ
4–septate, 20 per cent
5–septate, 14 per cent, 50 x 3.6 (43–60 x 3.1–4.4)μ

Average of the above measurements:
Conidia: 0–septate, 1 per cent
1–septate, 3 per cent
2–septate, 3 per cent
3–septate, 33 per cent
4–septate, 19 per cent
5–septate, 41 per cent, 51.2 x 4.3μ
6–septate, very rare

25. *Fusarium diplosporum* Cke. et Ell.

Cf. Cooke, M. C., and Ellis, J. B., Grevillea **7**:38. 1878. Saccardo, Syll. Fung. **4**:701. 1886.

The following description is from Saccardo:

" Roseum, pulvinatum; conidiis aliis fusiformibus, utrinque acutis, arcuatis, nucleatis, demum leniter 3–septatis 40μ long., aliis ellipticis, uniseptatis 18 x 8μ.

" Hab. In caulibus Solani tuberosi, New Jersey, Amer. bor."

The abbreviated, arthrosporial, uniseptate, conidia, 18 x 8μ in size, are so uncommon that there is no doubt in the writer's mind that the organism could easily be identified when found, and thus must be recognized as a good species. The organism was not isolated by the writer.

VII. SECTION SPOROTRICHIELLA Wr. (Fig. 1Q). Wollenweber, H. W., Maine Agr. Exp. Sta., Bul. 219:256. 1913

Fusaria of this section have from pyriform to nearly globular, mostly 0–septate, microconidia. Typical sickle-shaped, septate conidia always present, at least in young artificial cultures.

Only one species of this section is presented here. This species was twice isolated from rotted potato tubers, and thus, at least in a way, may be considered as of more or less common occurrence on this substratum. There are many other Fusaria of this section reported on such substrata as corn, carnations, apples, and others, a number of which are discussed by Lewis (1913). None of the species of this section mentioned by him, however, seem to be identical with the one here reported.

Lewis (1913:257) reports that the following organisms were isolated also from potatoes: (1) *F. Poae* (Peck) Wr., (2) *F. Solani* (Mart.) Ap. et Wr., (3) *F. conglutinans* Wr., (4) *F. Helianthi* Sacc. var., (5) *F. pirinum* (Fries) Sacc., and (6) *F. orthoceras* Ap. et Wr.

Numbers 2 and 6 are described in this paper; numbers 1 and 4 are closely related to *F. sporotrichioides* n. sp., and belong to section Sporotrichiella; number 3 belongs to section Elegans and differs from *F. orthoceras* by absence of red-wine color on rice (see Wollenweber, 1913 a:30); number 5 may belong to section Arthrosporiella.

No technical description, except results of inoculations — for potatoes always negative — and certain characters of color and of colony growth, is given, and thus a proper identification is rendered impracticable.

26. **Fusarium sporotrichioides** n. sp. (Figs. 1Q and 22; Pl. III, fig. 1)

Conidia scattered in aërial mycelium or in pseudopionnotes and distinct sporodochia; of diverse type, ranging from unicellular, more or less pyriform, microconidia of sporotrichial form, to sickle-shaped, 3– to 10–septate, apically pointed, pedicellate, macroconidia; 0–septate, sporotrichial conidia average 10.5 x 6 (9.5–11.4 x 5.6–6.5)μ; sickle-shaped, 3–septate conidia average 30.4 x 3.8μ, and 5–septate average 50.5 x 4.3μ; conidia of pseudopionnotes stage resemble those of *F. anguioides*; chlamydospores often present, intercalary, commonly in small clusters;

color of aërial mycelium and of substratum from white to clay (Pl. III, fig. 1) and pink, similar to the color of *F. bullatum* var. *roseum* (Pl. III, fig. 2).

Hab. On rotted tubers of *Solanum tuberosum,* together with *F. Solani* and *F. oxysporum,* New York State.

Latin description.—Conidiis in aerio mycelio sparsis vel in pseudopionnotibus et sporodochiis distinctis; typis variis: interdum microconidiis unicellularibus, plus minusve pyriformibus, sporotrichialibus; interdum macroconidiis falciformibus, 3–10–septatis, apice acutis, pedicellatis; conidiis 0–septatis sporotrichialibus plerumque 10.5 x 6 (9.5–11.4 x 5.6–6.5)μ; falciformibus, 3–septatis 30.4 x 3.8μ, et 5–septatis 50.5 x 4.3μ; conidiis pseudopionnotum gradu similibus conidiis F. anguioidum; chlamydosporis saepe visis, intercalaribus, plerumque minutis uveosis; aerio mycelio substratoque ex albo argillaceo vel rubello, simili colore F. bullati rosei.

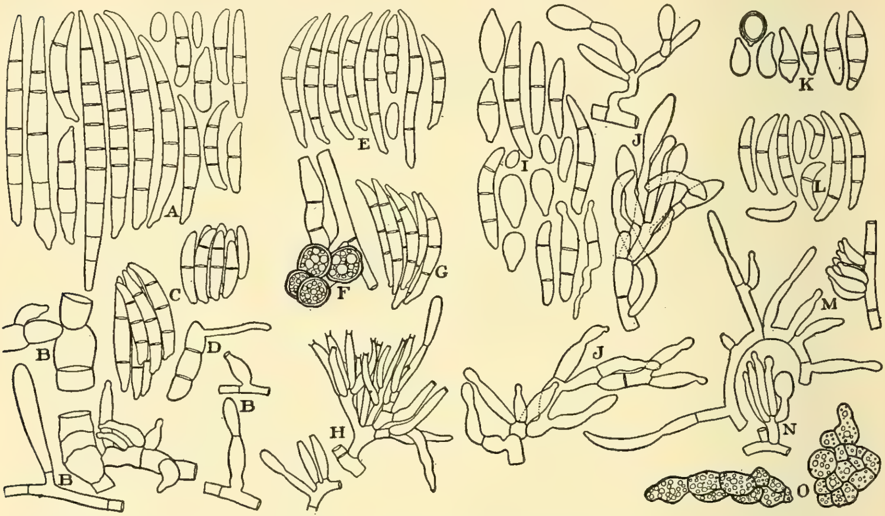

Fig. 22.—*Fusarium sporotrichioides.* A, *Pseudopionnotal conidia,* B, *various simple conidiophores,* C, *balls of conidia,* D, *germinating conidium, from 5-days-old culture on hard potato agar;* E, *sporodochial conidia,* F, *chlamydospores,* G, *ball of conidia,* H, *compound conidiophores, from 86-days-old culture on hard potato agar;* I, *sporotrichial conidia (some germinating),* J, *conidiophores, from aërial mycelium of 16-days-old culture on wheat grain;* K, *typical, thick-walled sporotrichial conidia,* L, *sickle-shaped conidia,* M, *conidiophores producing sickle-shaped conidia,* N, *conidiophore producing sporotrichial conidia,* O, *plectenchymic stroma, from 52-days-old culture on red raspberry cane plug*

Hab. In tuberibus putridis Solani tuberosi una cum F. Solani et F. oxysporo, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture five days old; conidia from pseudopionnotes:

Conidia: 0–septate, 2 per cent
1–septate, 10.5 per cent, 19 x 3.5μ
2–septate, 5.5 per cent, 28 x 3.7μ
3–septate, 37 per cent, 32.5 x 4.2 (24–50 x 3.9–4.7)μ
4–septate, 6 per cent, 45 x 4.2μ
5–septate, 33 per cent, 48 x 4.5 (40–62 x 3.9–5.3)μ
6– to 10–septate, 6 per cent, 50–90 x 5–6.1μ

On hard potato agar, culture eighty-six days old; conidia from a small sporodochium, semi-dry:

Conidia: 3–septate, nearly 100 per cent, 29.5 x 3.4 (20–41 x 3–4)μ

On red raspberry cane plug, culture fifty-two days old:

(1) Conidia of sporotrichial form

Conidia: 0–septate, 97 per cent, 9.5 x 6.5 (7–12 x 4–7.5)μ
1–septate, 3 per cent, 13.5 x 5.2 (10–15 x 4.8–6)μ
2–septate, rare
3–septate, exceptional, one measured, 21 x 5.2μ

(2) Conidia of sickle-shaped form

Conidia: 0–septate, 35 per cent, 11 x 2.9 (10–13 x 2.5–3.1)μ
1–septate, 60 per cent, 15.5 x 3.2 (12–22 x 2.9–3.5)μ
2–septate, very rare
3–septate, 5 per cent, 25 x 3.5 (20–28 x 3–4)μ

On hard lima-bean agar with 2 per cent glucose, culture 10 days old; conidia from a pseudopionnotes:

Conidia: 0–septate, 5 per cent, 10 x 3 (5.2–16 x 2.1–3.7)μ
1–septate, 15 per cent, 22 x 3.3 (16–28 x 3–3.5)μ
2–septate, 2 per cent
3–septate, 22 per cent, 33 x 3.8 (27–49 x 3.5–4.8)μ
4–septate, 23 per cent, 45 x 4 (38–49 x 3.9–4.1)μ
5–septate, 30 per cent, 51 x 4.1 (41–62 x 3.5–4.7)μ

6–septate, 1.5 per cent, 65 x 4.1 (60–80 x 3.7–4.4)μ (only four measured)
7–septate, 1.5 per cent, 67 x 4.5 (62–72 x 4.1–5.3)μ (only four measured)

On wheat grain culture sixteen days old; conidia from aërial mycelium:
(1) Sporotrichial form
Conidia: 0–septate, 70 per cent, 11.4 x 5.6 (8–16 x 4–6.5)μ
1–septate, 30 per cent, 14 x 5.6 (11–17 x 4.8–6)μ
(2) Sickle-shaped form
Conidia: 0–septate, 9 per cent, 10 x 3.2μ
1–septate, 68 per cent, 20 x 3.8 (12–24 x 3–4.2)μ
2–septate, 5 per cent
3–septate, 18 per cent, 32 x 4.3 (24–44 x 3.9–4.7)μ

Average of the above measurements:
(1) Sporotrichial form
Conidia: 0–septate, 83 per cent, 10.5 x 6μ
1–septate, 17 per cent, 14 x 5.4μ
2– and 3–septate, rare to very rare
(2) Sickle-shaped form
Conidia: 0–septate, 10 per cent
1–septate, 31 per cent, 10 x 3.1μ
2–septate, 2 per cent
3–septate, 36 per cent, 30.4 x 3.8μ
4–septate, 6 per cent
5–septate, 13 per cent, 50.5 x 4.3μ
6– to 10–septate, 2 per cent, about 67 x 4.5μ

VIII. Section **FERRUGINOSUM** n. sec.

Conidia mostly of an intermediate type, between that of section Gibbosum and section Roseum, 3– to 7–septate; intercalary chlamydospores typically present. Substratum and basal layer of aërial mycelium varying from white when young to different hues of red at maturity.

27. **Fusarium arcuosporum** n. sp. (Figs. 1B$_1$ and 23; Pl. ii, figs. 7 and 8; Pl. vi, fig. 10)

Conidia very gradually pointed toward apex, distinctly and often prominently pedicellate, typically much arcuate, 5-septate, 49.2 x 4 (42–54 x 3.6–4.2)μ, also often from 3- to 7-septate; single on aërial conidiophores and in from small to medium (up to 1.5 millimeters in diameter) sporodochia, the latter sometimes converging into pseudopionnotes; intercalary chlamydospores in mycelial threads typically present, though not numerous, and single or only in small clusters of from two to four cells; aërial mycelium usually well developed, uniform, white at first, then, on substrata poor in glucose, of different tints of pink with the substratum of a pomegranate color of different density; on potato agar rich in glucose, color as shown in Plate II, figures 7 and 8.

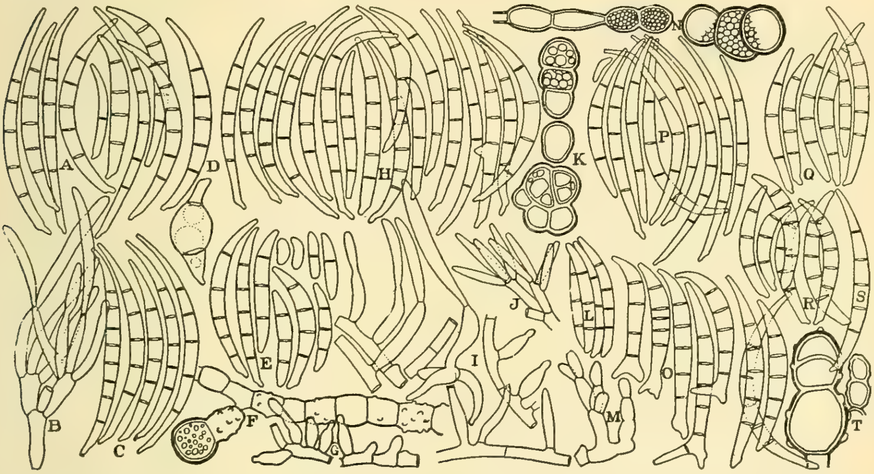

FIG. 23.—*Fusarium arcuosporum.* A, *Sporodochial conidia,* B, *conidiophore, from 20-days-old culture on hard lima-bean agar with 2 per cent glucose;* C, *conidia from small aërial sporodochium from 49-days-old culture on whole steamed potato tuber;* D, *typical conidium (the long one) and abnormal pseudopionnotal conidium from 11-days-old hard potato agar;* E, *aërial conidia,* F, *chlamydospores,* G, *conidiophores, from 75-days-old culture on red raspberry cane plug;* H, *pseudopionnotal conidia,* I, *conidiophores, from 12-days-old culture on slightly acidified hard potato agar;* J, *conidiophore,* K, *chlamydospores,* L, *conidial ball,* M, *conidiophores, from aërial mycelium from 75-days-old culture on red raspberry cane plug;* N, *chlamydospores from 173-days-old culture on corn agar;* O, *peculiarly branched,* P, *normal, pseudopionnotal conidia from 14-days-old culture on hard lima-bean agar;* Q, *conidia from aërial sporodochium of 107-days-old culture on potato stem plug;* R, *conidia from 109-days-old culture on rye straw;* S, *typical sporodochial conidium from 24-days-old culture on potato tuber plug;* T, *chlamydospores from 109-days-old culture on rye straw*

Hab. On rotted tubers of *Solanum tuberosum*, in association with *F. anguioides*, Castile, New York.

Latin description.—Conidiis maxime gradatim in apicem acutis, distincte vel saepe insignite pedicellatis, typice magnopere arcuatis, 5–septatis, 49.2 x 4 (42–54 x 3.6–4.2)μ, etiam saepe 3–7–septatis; continuis in aeriis conidiophoris vel minutis mediocribusve (usque ad $\frac{1}{5}$ mm. diam.) sporodochiis in pseudopionnotes interdum vergentibus; chlamydosporis intercalaribus sed non plurimis, continuis vel tantum minutis uveosis, 1–3–septatis; aerio mycelio fere plene maturo, uniformi, primum albo, dein, in substratis parum glucosis, rubello varie tincto, substrato "pomegranate" (R); in Solani tuberosi agare perglucoso, colore Tab. II, figg. 7, 8, exhibito.

Hab. In tuberibus putridis Solani tuberosi una cum F. anguioide, Castile, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture twelve days old; conidia from aërial mycelium:

Conidia: 0– and 1–septate, rare, immature
3–septate, 17 per cent, 43 x 3.5 (35–56 x 3–4.1)μ
4–septate, 19 per cent, 49 x 3.5 (38–59 x 3–4.1)μ
5–septate, 64 per cent, 54 x 3.7 (40–74 x 3–4.4)μ

On red raspberry cane plug, culture seventy-five days old; conidia from aërial mycelium:

Conidia: 3–septate, 10 per cent, 45 x 3.4 (28–51 x 3–4)μ
4–septate, 30 per cent, 50 x 3.5 (36–62 x 3–4.3)μ
5–septate, 60 per cent, 53 x 3.6 (44–62 x 3.5–4)μ

On hard lima-bean agar with 2 per cent glucose, culture seven days old; conidia from aërial mycelium:

Conidia: 3–septate, 30 per cent } (granulation of the protoplasm was too
4–septate, 15 per cent } great to clearly distinguish septation)
5–septate, 55 per cent, 53 x 3.8 (45–62 x 3.4–4.4)μ

On hard lima-bean agar with 2 per cent glucose, culture twenty days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, rare
4–septate, 4 per cent, 47 x 4.2μ (only two measured)
5–septate, 95 per cent, 54 x 4 (47–63 x 3.5–4.8)μ
6–septate, 1 per cent, 52 x 4.1μ (only two measured)

On potato tuber plug, culture twenty-four days old; conidia from small sporodochia:

Conidia: 1–septate, 1 per cent, about 14 x 3.5μ (only one measured)
3–septate, 10 per cent, 35 x 3.6 (27–43 x 3–4.4)μ
4–septate, 12 per cent
5–septate, 77 per cent, 45 x 4 (35–51 x 3.3–4.8)μ
6– and 7–septate, rare, 52 x 4.5μ (only two measured)

On potato stem plug, culture one hundred and seven days old; conidia from aërial mycelium:

Conidia: 0–septate, rare
1–septate, 2 per cent
2–septate, rare
3–septate, 50 per cent, 31 x 3.7μ
4–septate, 25 per cent, 40 x 4μ
5–septate, 23 per cent, 42 x 4.2 (35–48 x 3.9–4.7)μ

On whole steamed potato tubers, culture forty-nine days old:

(1) Conidia from numerous small sporodochia, close to the substratum

Conidia: 3–septate, 6 per cent, about 30 x 3.5 (only a few spores measured)
4–septate, 6 per cent
5–septate, 80 per cent, 46 x 4.1 (40–53 x 3.9–4.1)μ
6–septate, 5 per cent, 54 x 4.1 (42–65 x 4–4.7)μ
7–septate, 3 per cent, 59 x 4.2 (48–65 x 4.1–4.5)μ
10–septate, only one was observed, 58 x 4.3μ (the largest)

(2) Conidia from small sporodochia, in aërial mycelium

Conidia: 3–septate, rare
4–septate, rare
5–septate, nearly 100 per cent, 51 x 4 (45–55 x 3.6–4.3)μ
6–septate, rare

On medium soft agar, culture eleven days old; conidia from aërial mycelium close to substratum:

Conidia: 1–septate, 2 per cent, 24 x 3.2 (only a few measured)
3–septate, 8 per cent, 42 x 4μ (only a few measured)
4–septate, 10 per cent
5–septate, 77 per cent, 45 x 4.1 (41–62 x 3.1–4.8)μ
6–septate, 3 per cent, 49 x 4.3μ (only a few measured)

On hard lima-bean agar, culture fourteen days old; conidia from minute converging sporodochia close to substratum:

Conidia: 3–septate, 3 per cent
4–septate, 1 per cent
5–septate, 95 per cent, 49 x 4.2 (41–63 x 3.5–5.3)μ
6–septate, 1 per cent, 54 x 4.7μ (only a few measured)

Average of the above measurements:

Conidia: 0– to 2–septate, very rare
3–septate, 13 per cent, 38 x 3.5μ
4–septate, 13.5 per cent
5–septate, 72.5 per cent, 49.2 x 4μ
6– and 7–septate, 1 per cent, 53.6 x 4.3μ

The organism occupies an intermediate position between section Roseum and section Ferruginosum; its type of conidia is that of section Roseum, while because of its true chlamydospores of only an intercalary kind it belongs to the section Ferruginosum.

An especially striking peculiarity of *F. arcuosporum* is the frequent occurrence, especially in young cultures on various agars, of conidia with more or less distinct branches and knobs (Fig. 23 o).

28. **Fusarium ferruginosum** n. sp. (Figs. 1, h to j, and 24; Pl. iii, figs. 9 and 10; Pl. vi, fig. 2)

Conidia well developed, in from small to medium-sized (up to 2 millimeters in diameter) sporodochia or in pseudopionnotes, very gradually pointed toward apex, distinctly pedicellate, more or less strongly arcuate, and broader in the middle or in the lower third of their length, typically 3– to 5–septate; 5–septate average 45.3 x 4.2 (28–53 x 4.1–4.2)μ; conidia from aërial mycelium — sometimes also from sporodochia produced on very old agar cultures — typically 3–septate, 30.8 x 3.8μ, mostly apedicellate; conidia from nearly white to pale pink buff and deep vinaceous in color; intercalary chlamydospores in mycelium always present, often very numerous, in long chains and large clusters; aërial mycelium always well developed, high, often very dense, white at first changing to pink and then ferruginous when mature, the last-named color being due to production of great masses of chlamydospores; color of substratum, on potato agar rich in glucose, at first pink, then ochraceous tawny, and finally ferruginous and Hay's russet.

Hab. On rotted tubers of *Solanum tuberosum*, Long Island, New York, on *Lycopersicum esculentum*, Virginia, and on *Panax quinquefolium*, New York State.

Latin description.—Conidiis plene maturis—sporodochiis minutis vel mediocribus (usque ad 2 mm. diam.) vel pseudopionnotibus — maxime gradatim in apicem acutis, distincte pedicellatis, plus minusve valide arcuatis, latioribus in medio vel in inferiore tertio longitudinis, typice 3–5-septatis; 5-septatis plerumque 45.3 x 4.2 (28–53 x 4.1–4.2)μ; conidiis in aerio mycelio — interdum etiam ex sporodochiis in vetustissimis agaris culturis crescentibus — typice 3-septatis, 30.8 x 3.8μ, plerumque apedicellatis; conidiis ex albido pallide rubellis, gilvis atque vinaceosis; chlamydosporis intercalaribus semper in mycelio praesentibus, saepe plurimis, longis catenulatis longis uveosisque; aerio mycelio semper plene maturo, alto, semper densissimo, primum albo, dein rubello demum ferrugineo, quem colorem magnae moles chlamydosporum ortae efficiunt; substrato in agare Solani tuberosi glucoso, primum rubello, dein "ochraceous tawny" (R), demum ferrugineo et "Hay's russet" (R).

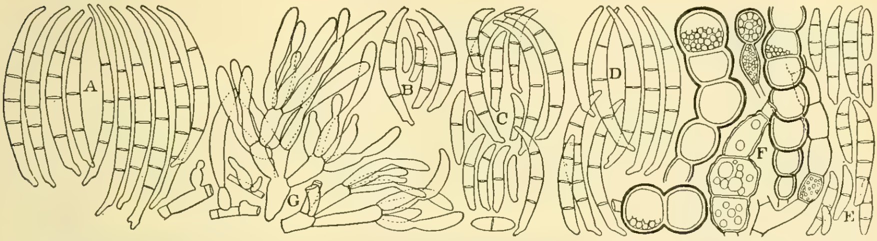

FIG. 24.—*Fusarium ferruginosum.* A, *Pseudopionnotal conidia from 10-days-old culture on hard lima-bean agar;* B, *sporodochial conidia from 130-days-old culture on hard lima-bean agar with 2 per cent glucose;* C, *aërial conidia from 14-days-old culture on slightly acidified hard potato agar;* D, *pseudopionnotal conidia from 3-days-old culture on hard lima-bean agar;* E, *aërial conidia,* F, *chlamydospores, from 35-days-old culture on corn meal;* G, *conidiophores from 3-days-old culture on hard lima-bean agar*

Hab. In tuberibus putridis Solani tuberosi, Long Island, New York, in Lycopersico esculento, Virginia, et in Panaci quinquefolio, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On hard lima-bean agar with 2 per cent glucose, culture one hundred and thirty days old; conidia from small sporodochia produced after the culture was over eighty days old:

Conidia: 0–septate, very rare, 9 x 2.8μ
1–septate, 4 per cent, 12 x 3.2μ
2–septate, 3 per cent, 17 x 3.5μ
3–septate, 93 per cent, 27.3 x 4.1 (17–33 x 3.8–4.4)μ

On hard potato agar with 10 per cent glucose, culture seven days old in poured plate:

(1) Chlamydospores
0–septate, 10 x 9.5 (7–12.5 x 7–11.5)μ
1–septate, 16 x 11μ
(2) Conidia from aërial mycelium
Conidia: 1–septate, 1 per cent
2–septate, 1 per cent
3–septate, 88 per cent, 32 x 3.7 (25–40 x 3.1–4.2)μ
4–septate, 6 per cent
5–septate, 4 per cent, 38 x 4.1 (35–42 x 3.9–4.4)μ

On hard lima-bean agar, culture three days old; conidia from pseudopionnotes (near the center of inoculation):

Conidia: 3–septate, 66 per cent, 36 x 3.9 (30–41 x 3.5–4.3)μ
4–septate, 20 per cent
5–septate, 14 per cent, 45 x 4.2 (42–56 x 3.9–4.7)μ

On corn meal agar, culture thirty-five days old:

(1) Chlamydospores typically in chains, 8.5 (5.8–15)μ in diameter
(2) Conidia from aërial mycelium
Conidia: 0–septate, 20 per cent, 8.8 x 2.6 (7–12 x 2.4–3)μ
1–septate, 46 per cent, 12.8 x 2.9 (9–19 x 2.5–3.1)μ
2–septate, 6 per cent
3–septate, 26 per cent, 20.4 x 3.4 (17–30 x 3–3.8)μ
4–septate, 2 per cent, 26.7 x 3.9 (24–29 x 3.7–4)μ

On hard lima-bean agar, culture ten days old; conidia from pseudopionnotes:

Conidia: 3–septate, 8 per cent, 41 x 3.9μ
4–septate, 12 per cent, 45.5 x 4μ

5–septate, 80 per cent, 53 x 4.2 (50–58 x 4.1–4.4)μ, the largest observed being 65 x 4.1μ

On slightly acidified hard potato agar, culture fourteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 26 per cent, 10 x 2.5 (8–14 x 1.7–3.5)μ
1–septate, 41.5 per cent, 17.3 x 3 (14–20 x 2.5–3.5)μ
2–septate, 10 per cent, 19.5 x 3.2 (15–31 x 2.9–4)μ
3–septate, 17.5 per cent, 28 x 3.6 (19–42 x 2.9–4.7)μ
4–septate, 3 per cent } 38 x 4.2 (30–45 x 3.9–5.2)μ
5–septate, 2 per cent }

Average of the above measurements:

Conidia: 0–septate, 7.5 per cent, 9.4 x 2.55μ
1–septate, 14.5 per cent, 13 x 3μ
2–septate, 3 per cent
3–septate, 48 per cent, 30.8 x 3.8μ
4–septate, 10 per cent, 38.8 x 4.03μ
5–septate, 17 per cent, 45.3 x 4.2μ

The organism was isolated only once from potato tubers, but it was isolated by the writer from a specimen of Rhizoctonia-infected tomato plant also, from Virginia, and by C. O. Dalrymple from ginseng garden soil in New York State. This seems to be a cosmopolitan species.

29. **Fusarium sanguineum** n. sp. (Fig. 25; Pl. III, figs. 7 and 8; Pl. VI, fig. 1)

Conidia typically strongly arcuate, gradually pointed toward apex, distinctly pedicellate, 3– to 5–septate — 3–septate conidia averaging 33.5 x 3.6 (24–37 x 3.4–3.8)μ and 5–septate averaging 45.2 x 4.1 (40–47 x 3.9–4.2)μ — single, in from small to medium-sized (up to 2 millimeters in diameter) sporodochia and in pseudopionnotes, the latter form of fructification dominant on most media, especially on agars; chlamydospores almost always present, intercalary in conidia and in mycelial threads, borne singly, in chains and in clusters; aërial mycelium seldom well developed and then from white to different shades of pink, on various agars mostly absent leaving exposed pseudopionnotes of ox-blood red color.

Hab. On rotted tubers of *Solanum tuberosum* in association with *F. lutulatum* var. *zonatum*, Ithaca, New York.

Latin description.—Conidiis typice valide arcuatis, gradatim in apicem acutis, distincte pedicellatis, 3–5–septatis — conidiis 3–septatis, 33.5 x 3.6 (24–37 x 3.4–3.8)μ, conidiis 5–septatis, 45.2 x 4.1 (40–47 x 3.9–4.2)μ — continuis, minutis vel mediocribus (usque ad 2 mm. diam.) sporodochiis et pseudopionnotibus, quo fructificationis modo in plurimis mediis frequentissimo imprimis in agaribus; chlamydosporis plerumque praesentibus, intercalaribus in conidiis et in hyphis, singulatim ortis, catenulatis vel uveosis; aerio mycelio raro plene maturo, sed, cum plene maturum, tum ex albo varie rubello tincto, in agaribus variis plerumque absente, pseudopionnotes "ox-blood red" (R) exponente.

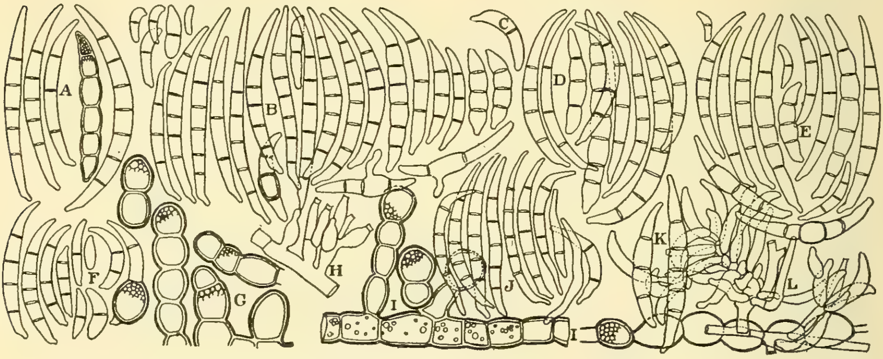

Fig. 25.—*Fusarium sanguineum.* A, *Pseudopionnotal conidia (one chlamydospore-like conidium) from 8-days-old culture on hard lima-bean agar with 2 per cent glucose;* B, *pseudopionnotal conidia from 14-days-old culture on hard lima-bean agar;* C, *typical conidium from 47-days-old culture on whole grains of wheat;* D, *pseudopionnotal conidia from 19-days-old culture on slightly acidified hard potato agar;* E, *pseudopionnotal conidia from 23-days-old culture on red raspberry cane plug;* F, *conidia,* G, *chlamydospores, from 76-days-old culture on potato tuber plug;* H, *conidiophores from 23-days-old culture on red raspberry cane plug;* I, *chlamydospores from 176-days-old culture on corn agar;* J, *sporodochial conidia from 41-days-old culture on rye straw;* K, *conidia from 176-days-old culture on corn meal;* L, *conidiophores from 47-days-old culture on whole grains of wheat*

Hab. In tuberibus putridis Solani tuberosi una cum F. lutulato var. zonato, Ithaca, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On red raspberry cane plug, culture twenty-three days old:
(1) Conidia from pseudopionnotes
Conidia: 1–septate, 7 per cent, 15 x 2.3 (10–23 x 2.2–2.5)μ
2–septate, 2.5 per cent
3–septate, 17 per cent, 32 x 3.4 (22–43 x 2.8–4.1)μ
4–septate, 7 per cent, 40 x 3.8 (35–45 x 3–4.3)μ
5–septate, 63.5 per cent, 47 x 4.2 (38–51 x 3.9–4.3)μ
6–septate, 3 per cent, 52–60 x 4.1–4.4μ (only a few measured)
7–septate, very few, 52–60 x 4.1–4.4μ (only a few measured, the thickest, swollen, 6.2μ in diameter)
(2) Chlamydospores
(a) Unicellular, in conidia, 6–12.5 x 6–8μ
(b) Unicellular, in mycelium, 8–17 x 7–14μ

On slightly acidified hard potato agar, culture nineteen days old; conidia from pseudopionnotes:
Conidia: 1–septate, 1 per cent
2–septate, 2 per cent
3–septate, 57 per cent, 37.5 x 3.6 (32–50 x 3–4)μ
4–septate, 17 per cent, 44 x 3.7 (38–60 x 3.5–4.4)μ
5–septate, 20 per cent, 45 x 4.1 (40–60 x 3.8–4.4)μ
$\left.\begin{array}{l}\text{6–septate}\\ \text{7–septate}\end{array}\right\}$ 3 per cent

On potato tuber plug, culture seventy-six days old; conidia from aërial mycelium:
Conidia: 0–septate, 5 per cent
1–septate, 15 per cent
2–septate, 10 per cent
3–septate, 55 per cent, 24 x 3.5 (17–35 x 3–4)μ
4–septate, 7 per cent
5–septate, 8 per cent, 40 x 4.1 (35–43 x 3.5–4.4)μ

On hard lima-bean agar with 2 per cent glucose, culture eight days old; conidia from pseudopionnotes:
Conidia: 3–septate, 3 per cent, 41 x 3.8 (35–44 x 3.5–4.1)μ
4–septate, 5 per cent
5–septate, 90 per cent, 47 x 4.2 (43–51 x 3.9–4.7)μ
6–septate, 2 per cent

On hard lima-bean agar, culture sixteen days old, most of the conidia swollen (only normal, not swollen, conidia, were measured):

Conidia: 1–septate, 9 per cent, 14.7 x 2.5 (12–19.5 x 2.3–3)μ
2–septate, very rare
3–septate, 37 per cent, 33 x 3.5 (18–41 x 3–4.1)μ
4–septate, 14 per cent
5–septate, 29 per cent, 47 x 3.9 (38–56 x 3.5–4.4)μ
6–septate, 7 per cent, 50 x 4 (43–58 x 3.9–4.4)μ
7–septate, 4 per cent, 50 x 4.1 (49–56 x 4–4.3)μ
8–septate, exceptional, 61 x 4.4μ (only one found and measured)

Average of the above measurements:

Conidia: 0–septate, 1 per cent
1–septate, 6 per cent
2–septate, 3 per cent
3–septate, 34 per cent, 33.5 x 3.6μ
4–septate, 10 per cent
5–septate, 42 per cent, 45.2 x 4.1μ
6– and 7–septate, 4 per cent, 50 x 4.05μ
8–septate, very rare, 61 x 4.4μ (only one measured)

30. *Fusarium sanguineum* var. **pallidium** n. var. (Figs. 1K and 26; Pl. III, figs. 5 and 6; Pl. VII, fig. 7)

Differs from *F. sanguineum* by better development of mycelium, by much slower rate of colony growth, by paler color of substratum and conidia (Pl. III, figs. 5 and 6), and by chlamydospores which are intercalary, as in *F. sanguineum*, but mostly in conidia and not in mycelium, and of much rarer occurrence than in *F. sanguineum*.

Hab. On rotted tubers of *Solanum tuberosum*, in association with *F. oxysporum*, South Dakota.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture three days old; conidia from pseudopionnotes:

Conidia: 2–septate, rare
3–septate, 53 per cent, 45 x 3.9 (38–56 x 3.5–4.1)μ
4–septate, 20 per cent, 40 x 4 (40–57 x 3.9–4.1)μ
5–septate, 20 per cent, 53 x 4.1 (47–60 x 3.9–4.3)μ
6– and 7–septate, 7 per cent, 55 x 4.3 (52–61 x 4.1–4.8)μ

On red raspberry cane plug, culture thirty-two days old; conidia from aërial mycelium:

Conidia: 1–septate, few
3–septate, 43 per cent, 30 x 3.8 (19–42 x 2.6–4.3)μ
4–septate, 36 per cent, 36 x 4 (31–42 x 3.5–4.3)μ
5–septate, 21 per cent, 39 x 4.1 (33–43 x 3.9–4.7)μ

On hard lima-bean agar with 2 per cent glucose, culture eight days old; conidia from pseudopionnotes:

Conidia: 3–septate, 60 per cent, 41 x 3.7 (35–48 x 3.5–4.1)μ
4–septate, 25 per cent, 51 x 3.8 (44–60 x 3.5–4.1)μ
5–septate, 15 per cent, 55 x 3.8 (52–56 x 3.5–4)μ

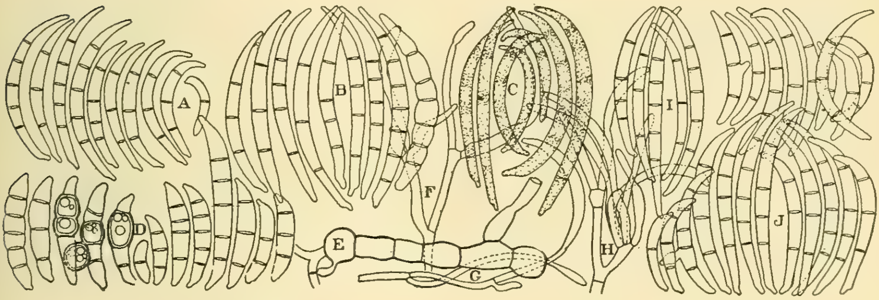

Fig. 26.—*Fusarium sanguineum var. pallidium.* A, *Pseudopionnotal conidia from 32-days-old culture on red raspberry cane plug;* B, *pseudopionnotal conidia from 3-days-old culture on slightly acidified hard potato agar;* C, *pseudopionnotal conidia with densely granulated content and indistinct septation from 8-days-old culture on hard lima-bean agar with 2 per cent glucose:* D, *conidia (normal, swollen, and with chlamydospores) from aërial mycelium from 35-days-old culture on whole steamed potato tubers;* E, *chlamydospore-like swellings in mycelium from 176-days-old culture on corn agar;* F, G, H, *conidiophores from 14-days-old culture on hard lima-bean agar;* I, *pseudopionnotal conidia from 35-days-old culture on whole steamed potato tubers;* J, *pseudopionnotal and aërial conidia from 14-days-old culture on hard lima-bean agar*

On whole steamed potato tuber, culture thirty-eight days old:

(1) From a tuft of conidiophores over dry surface of the tuber;[44] type of spores very much like that of *F. discolor* var. *triseptatum*

Conidia: 1–septate, 2 per cent
2–septate, 7 per cent, about 17 x 3.6μ
3–septate, 72 per cent, 24 x 4.5 (19–32 x 4–4.8)μ

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[44] This type is very different from the general type, and therefore was not taken into account for average size of conidia.

4–septate, 15 per cent, 26 x 4.7 (24–30 x 4.6–5.2)μ
5–septate, 4 per cent, 28 x 4.7 (26–32 x 4.2–5.2)μ, the largest 45.5 x 5.2μ

(2) From pionnotes over the cut surface of the tuber; spore type close to that of *F. arcuosporum*

Conidia: 3–septate, 68 per cent, 41 x 3.2 (29–48 x 2.9–3.5)μ
4–septate, 20 per cent, 44 x 3.4 (36–51 x 3.2–4)μ
5–septate, 12 per cent, 48 x 3.4 (40–57 x 3–3.7)μ

On hard lima-bean agar, culture fourteen days old; conidia from pseudopionnotes:

Conidia: 1–septate, 2 per cent, 21 x 2.8μ (only three measured)
2–septate, exceptional
3–septate, 83 per cent, 37 x 3.5 (33–42 [61 exceptional] x 3–3.9)μ
4–septate, 12 per cent
5–septate, 3 per cent, 47 x 3.8 (42–60 x 3.5–4)μ

On the same medium and of the same age as the above, but from a semi-dry minute sporodochium near the upper margin of the slant:

Conidia: 1–septate, 1 per cent
3–septate, 56 per cent, 43 x 3.8 (37–48 x 3.5–4.1)μ
4–septate, 29 per cent
5–septate, 14 per cent, 44 x 3.9 (40–49 x 3.5–4.2)μ

31. **Fusarium bullatum** n. sp. (Figs. $1A_1$ and 27)

Conidia typically somewhat less arcuate than the other species of the same section, less pointed toward the apex, and broader, usually distinctly pedicellate, mostly 5–septate, 42 x 4.3 (31–47 x 4.1–4.9)μ, from pale cream to salmon in color; chlamydospores intercalary in mycelium, mostly in chains and from small to large clusters; aërial mycelium nearly always well developed, of uniform medium height and density, nearly pure white in color; substratum on various agars from colorless to a tint of light buff.

Hab. On rotted tuber of *Solanum tuberosum*, together with *F. bullatum* var. *roseum* and with *F. oxysporum* var. *resupinatum*, in Iowa.

Latin description.—Conidiis typice paulo minus arcuatis quam ceteris ejusdem sectionis speciebus, minus in apicem acutis, latioribus, plerumque distincte pedicellatis, plerumque 5–septatis, 42 x 4.3 (31–47 x 4.1–4.9)μ, e pallide "cream color" (R) "salmon color" (R); chlamydosporis intercalaribus in mycelio, plerumque catenulatis, parvis vel magnis uveosis; aerio mycelio semper plene maturo, uniformi mediocri altitudine atque densitate, ferme pure albo; substrato in agaribus variis ex hyalino pallide luteolo tincto.

Hab. In tuberibus putridis Solani tuberosi una cum F. bullato var. roseo et cum F. oxysporo var. resupinato, Iowa, Amer. bor.

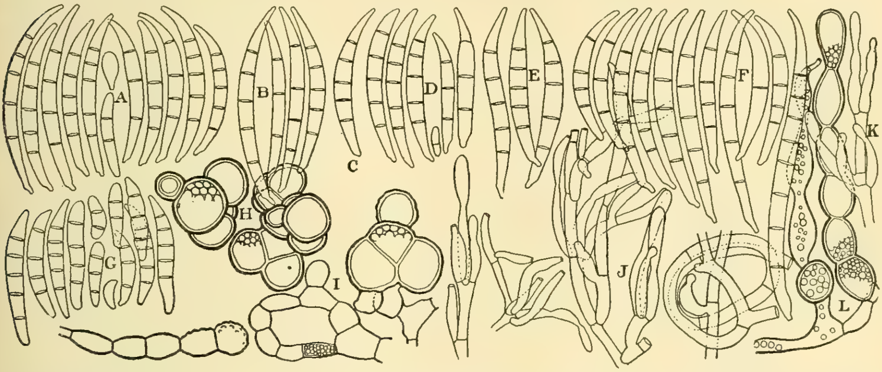

FIG. 27.—*Fusarium bullatum.* A, *Pseudopionnotal conidia from 10-days-old culture on hard lima-bean agar;* B, *pseudopionnotal conidia from 77-days-old culture on red raspberry cane plug;* C, *a typical conidium from 51-days-old culture on potato tuber plug;* D, *conidia from 76-days-old culture on potato tuber plug;* E, *conidia from 19-days-old culture on hard lima-bean agar;* F, *pseudopionnotal conidia from 7-days-old culture on hard potato agar;* G, *conidia,* H, *chlamydospores, from 42-days-old culture on rye straw;* I, *chlamydospores from 77-days-old culture on red raspberry cane plug;* J, *conidiophores from 7-days-old culture on hard potato agar;* K, *conidiophore from 10-days-old culture on hard lima-bean agar;* L, *chains of chlamydospores from 175-days-old culture on corn agar*

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture seven days old; conidia from pseudopionnotes:

Conidia: 3–septate, 16 per cent, 36 x 3.8 (29–50 x 3.5–4.9)μ
4–septate, 19 per cent, 43 x 4.1 (33–50 x 3.9–4.9)μ
5–septate, 65 per cent, 45 x 4.4 (37–63 x 4.1–4.9)μ
6– to 8–septate, rare, 65 x 4.9 (60–82 x 4.7–5.3)μ

On red raspberry cane plug, culture seventy-seven days old; conidia from aërial mycelium close to substratum:

Conidia: 3–septate, 25 per cent
4–septate, 20 per cent
5–septate, 55 per cent, 48 x 4.1 (42–53 x 3.5–4.4)μ

On potato tuber plug, culture seventy-six days old; conidia from aërial mycelium:

Conidia: 1–septate, 5 per cent
2–septate, 10 per cent
3–septate, 45 per cent, 31 x 3.7 (22–39 x 3.5–4.6)μ
4–septate, 20 per cent
5–septate, 20 per cent, 39 x 4.2 (35–45 x 4–4.4)μ

Same as preceding, culture eighty days old; conidia from aërial mycelium:

Conidia: 0–septate, 1 per cent
1–septate, 4 per cent
2–septate, 1 per cent
3–septate, 40 per cent, 30 x 3.7 (22–39 x 3.1–4.1)μ
4–septate, 20 per cent
5–septate, 34 per cent, 41 x 4.2 (35–49 x 3.5–4.7)μ
6– to 9–septate, rare (the largest, 9–septate, 56 x 5.7μ)

On hard lima-bean agar with 2 per cent glucose, culture nineteen days old; conidia from pseudopionnotes:

Conidia: 3–septate, 2 per cent, 35 x 3.8μ (only three measured)
4–septate, 1 per cent, 38 x 3.9μ (only four measured)
5–septate, 97 per cent, 47 x 4.4 (43–54 x 4–4.7)μ
6–septate, rare, 51 x 4.5μ (only three measured, 48–53 x 4.3–4.8μ)

On potato stem plug, culture one hundred and fourteen days old; conidia from aërial mycelium:

Conidia: 3–septate, 16 per cent
4–septate, 14 per cent
5–septate, 70 per cent, 41 x 4.35 (36–46 x 4–4.7)μ

On hard lima-bean agar, culture ten days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, 5 per cent
2–septate, rare
3–septate, 15 per cent
4–septate, 10 per cent
5–septate, 70 per cent, 43 x 4.1 (35–50 x 3.8–4.7)μ

In some cases 5–septate conidia were up to 100 per cent, in others conidia of lower septation were as high as 50 per cent. Of the smaller, 3–septate conidia were dominant.

On rye straw, culture fifty days old; conidia from aërial mycelium close to substratum:

Conidia: 3–septate, 10 per cent
4–septate, 5 per cent
5–septate, 85 per cent, 31.25 x 4.1 (27–36 x 3.9–4.6)μ

Average of the above measurements:

Conidia: 0–septate, rare
1–septate, 2 per cent
2–septate, 1.5 per cent
3–septate, 21 per cent, 33 x 3.75μ
4–septate, 15 per cent
5–septate, 60.5 per cent, 42 x 4.3μ
6– to 8–septate, very rare, 58 x 4.7μ

32. *Fusarium bullatum* var. **roseum** n. var. (Fig. 28; Pl. III, fig. 2)

Differs from *F. bullatum* mainly by its red substratum (on agar rich in glucose, see Plate III, figure 2) and by higher septation of conidia.

Hab. Same as that of *F. bullatum.*

This organism and *F. bullatum* are in general, especially in minute details of the character of the mycelium and in the chlamydospores, very much alike, and both were isolated from the same planting of diseased tissue of a rotted potato tuber and separated out on the first dilution. It is possible that they represent an example of a sudden and permanent variation of one Fusarium in pure culture.

IX. Section Elegans Wr., Phytopath. 3:28, fig. 1, e, f, s, t, u, v. 1913

Fusaria with ellipsoidal, 0– and 1–septate, microconidia; macroconidia typically 3–septate, often also 4– or 5–septate, more or less gradually pointed toward apex, pedicellate; chlamydospores intercalary and terminal in and on mycelial hyphæ, always present, and often also in and on conidia: color of conidia mostly pinkish buff; color of substratum, and of aërial mycelium when present (on potato agar rich in glucose and on rice), typically of various vinaceous hues, from light pinkish to dense purple.

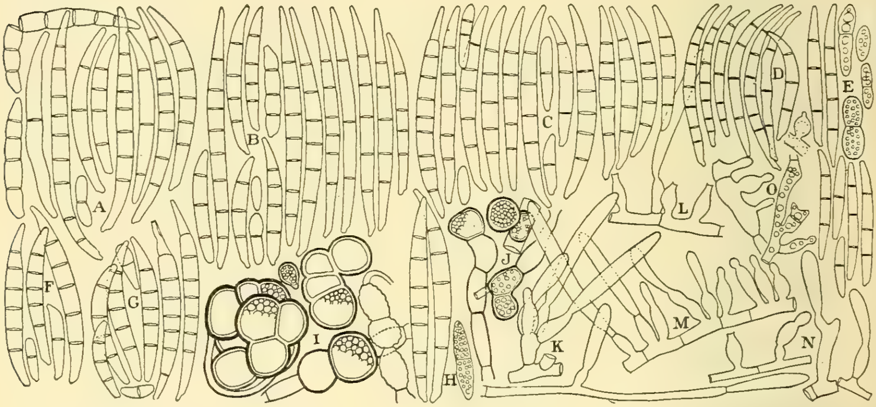

Fig. 28.—*Fusarium bullatum var. roseum.* A, *Pseudopionnotal conidia from 7-days-old culture on slightly acidified hard potato agar;* B, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar;* C, *pseudopionnotal conidia from 12-days-old culture on hard lima-bean agar;* D, *sporodochial conidia from 71-days-old culture on potato stem plug;* E, *pseudopionnotal conidia (many are swollen and with oil drops) from 35-days-old culture on corn meal;* F, *conidia from 37-days-old culture on hard oat gar;* G, *conidia from 67-days-old culture on red raspberry cane plug;* H, *pseudopionnotal conidia from 16-days-old culture on hard lima-bean agar (the two at the left typical for older growth, the one at the right for young growth);* I, *chlamydospores, intercalary in chains and in clusters, from 67-days-old culture on red raspberry cane plug;* J, *chlamydospores from 12-days-old culture on hard lima-bean agar;* K, L, M, *conidiophores from 7-days old culture on slightly acidified hard potato agar;* N, *conidiophores from 12-days-old culture on hard lima-bean agar;* O, *conidiophores from 35-days-old culture on corn meal*

33. *Fusarium orthoceras* Ap. et Wr. (Figs. 1, I_1 to K_1, and 29)

Appel, O., and Wollenweber, H. W. Arb. K. biol. Anst. Land- u. Forstw. 8:141–156; Pl. I, figs. 60 to 64; Pl. III, fig. 2. 1910. Wollenweber, H. W., Phytopath. 3:30, fig. 1s. 1913.

Microconidia always greatly in excess; macroconidia ranging from rare to several per cent of the total number of conidia, mostly nearly straight, sometimes slightly curved, typically 3–septate, 36 x 3.85 (25–40 x 3.2–4)μ; aërial mycelium usually well developed, from white to a tint of olive-buff; substratum, on potato agar rich in glucose, colorless at first, then from russet vinaceous to deep brownish vinaceous; no sporodochia; no pseudopionnotes; no sclerotia. Not the same as *F. oxysporum.*

Hab. Roots and tubers of Solanaceæ, also on various other hosts and in soil, in Europe and North America.

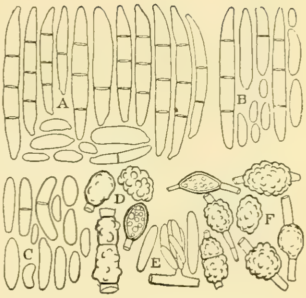

Fig. 29.—*Fusarium orthoceras.* A, *Conidia from 18-days-old culture on slightly acidified hard potato agar;* B, *conidia from 26-days-old culture on potato tuber plug;* C, *conidia from 15-days-old culture on hard lima-bean agar with 2 per cent glucose;* D, *chlamydospores from 18-days-old culture on slightly acidified hard potato agar;* E, *conidia, some in a ball, with conidiophore of common type, from 30-days-old culture on corn agar;* F, *chlamydospores from 53-days-old culture on potato tuber plug*

The organism was not isolated by the writer. The original culture was obtained from the Centralbureau der Association Internationale des Botanistes, Amsterdam, Holland, and was studied in conjunction with the other Fusaria. The above description is in the main the same as that given by Wollenweber.

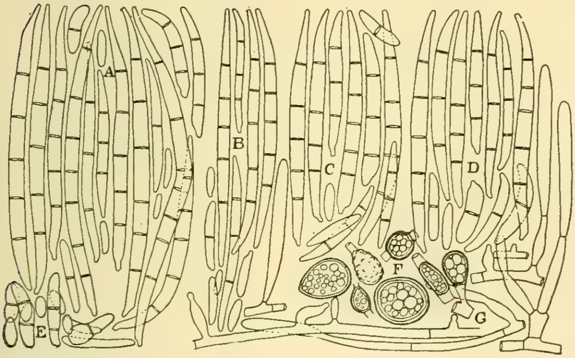

Fig. 30.—*Fusarium angustum.* A, *Pseudopionnotal conidia from 4-days-old culture on slightly acidified hard potato agar;* B, *conidiophores from surface of the exposed substratum from 25-days-old culture on corn agar;* C, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar with 2 per cent glucose;* D, *pseudopionnotal conidia from 8-days-old culture on hard lima-bean agar;* E, *microconida from aërial mycelium;* F, *chlamydospores from 74-days-old red raspberry cane plug;* G, *conidiophores from 9-days-old culture on hard lima-bean agar with 2 per cent glucose*

34. **Fusarium angustum** n. sp. (Figs. 1, G_1 and H_1, and 30)

Conidia gradually pointed toward apex, from slightly curved to nearly straight or anguiform, usually distinctly pedicellate, mostly 3–septate, 45.64 x 3.52 (42–49 x 3.3–3.6)μ, often 0– to 5–septate, sometimes 6– to 8–septate; on various agars usually producing thin pseudopionnotes, otherwise very similar to *F. oxysporum.*

Hab. In discolored fibrovascular bundles of tubers of *Solanum tuberosum,* Ithaca, New York.

Latin description.—Conidiis gradatim in apicem acutis, paulum curvatis vel prope rectis anguiformibusque, plerumque distincte pedicellatis, plerumque 3–septatis, 45.64 x 3.52 (42–49 x 3.3–3.6)μ, saepe 0–5–septatis, interdum 6–8–septatis; in variis agaribus tenues pseudopionnotes plerumque exhibentibus, aliter simillimis F. oxysporo.

Hab. In tuberum fasciculis decoloratis fibro-vascularibusque Solani tuberosi, Ithaca, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture four days old; conidia from pseudopionnotes:

Conidia: 0–septate, 6 per cent, 12 x 2.5 (8–18 x 1.8–3.5)μ
1–septate, 7 per cent, 32 x 2.9 (19–52 x 2.5–4.2)μ
2–septate, 2 per cent, 32 x 2.7 (30–39 x 2.5–3)μ
3–septate, 44 per cent, 42.2 x 3.3 (31–69 x 2.5–4.4)μ
4–septate, 13 per cent, 60.4 x 3.8 (47–70 x 3.5–4.4)μ
5–septate, 27 per cent, 63 x 3.9 (43–81 x 3.5–4.1)μ
6– to 8–septate, 1 per cent, 90 x 4.1 (80–102 x 4–4.4)μ

On red raspberry cane plug, culture seventy-four days old:

Conidia: 0–septate, 90 per cent
1–septate, 7 per cent
3–septate, 3 per cent, 39.4 x 3.81 (30–50 x 3–4.8)μ

On hard lima-bean agar with 2 per cent glucose, culture nine days old; conidia from pseudopionnotes:

Conidia: 0–septate, 54 per cent, 10 x 2.8 (5–15 x 2.7–3.1)μ
1–septate, 7 per cent, 18 x 2.9 (15–22 x 2.7–3.1)μ
2–septate, 3 per cent
3–septate, 20 per cent, 43 x 3.6 (29–53 x 3–4.1)μ
4–septate, 8 per cent
5–septate, 8 per cent, 59 x 4.5 (52–70 x 3.9–4.8)μ
6–septate, few, 70 x 4.7μ (only one measured)

On hard lima-bean agar, culture eight days old; conidia from pseudopionnotes (measurements made in April):

Conidia: 0–septate, 39 per cent, 11 x 2.6 (8.7–14 x 2.1–3.2)μ
1–septate, 17 per cent, 18 x 3.2 (14–23 x 2.9–3.9)μ
2–septate, 3 per cent, 24 x 3.3μ (only four measured)
3–septate, 32 per cent, 49 x 3.6 (42–55 x 3.5–4.8)μ
4–septate, 2 per cent, 53 x 4μ (only four measured)
5–septate, 6 per cent, 58 x 4.3 (52–65 x 3.5–4.8)μ
6–septate, 1 per cent, 73 x 4.7μ (only one measured)

On same media, culture nine days old (measurements made in May):

Conidia: 0–septate, 11 per cent, 10 x 2.5 (5–15 x 1.6–3.3)μ
1–septate, 9 per cent, 17 x 3.1 (12–24 x 2.6–4)μ
2–septate, 1 per cent
3–septate, 43 per cent, 49 x 3.6 (31-54 x 2.7–4.1)μ
4–septate, 13 per cent
5–septate, 23 per cent, 50 x 4 (54–71 x 3.5–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 27.5 per cent, 11 x 2.6μ
1–septate, 10 per cent, 21 x 3μ
2–septate, 2 per cent, 28 x 3μ
3–septate, 35 per cent, 45.64 x 3.52μ
4–septate, 9 per cent, 56.7 x 3.9μ
5–septate, 16 per cent, 60 x 4.2μ
6–septate, 0.5 per cent, 78 x 4.5μ
8–septate, very rare, 78 x 4.5μ

35. *Fusarium redolens* Wr.[45] var. **Solani** n. var. (Figs. 1P$_1$ and 31; Pl. II, figs. 3 and 4; Pl. V, fig. 2)

Macroconidia typically more or less gradually attenuate, sometimes suddenly constricted at the apex, pedicellate, typically broader toward and more curved near apex, 3–septate, 36.4 x 4.86 (31–41 x 4.3–5)μ, often also 4– and 5–septate, usually in numerous from small to medium (up to 2½ millimeters, commonly ½ millimeter, in diameter) sporodochia, sometimes, especially on different agars and in an early stage of culture growth, in pseudopionnotes; from nearly white in color when in small powdery masses, to a bright orange color when in comparatively large sporodochia on nearly dry potato stems; mostly, however, from a light pinkish cinnamon to a pinkish buff; aërial mycelium sometimes medium well developed but usually very scant, short, from white to somewhat grayish or brownish gray in color (Pl. II, fig. 3); color of substratum, on agar rich in glucose, as shown on Plate II, figure 4, which is different from all the other Fusaria of the section Elegans.

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[45] *Fusarium redolens* Wr. is described by its author (see Wollenweber, Phytopath. **3**: 29–30, fig. 1 E, 1913) as follows: "Differs from all the above-mentioned species [*F. oxysporum*, *F. tracheiphilum*, *F. vasinfectum*, *F. vasinfectum* var. *inodoratum*, *F. lycopersici*, and *F. niveum*] in the large size of its triseptate conidia, 30–40 x 4.5–5.5μ, and in the color of the brownish white conidial masses. A lilac odor is produced on rice and milk. No blue sclerotia. Vascular parasite, cause of wilt and foot disease of *Pisum sativum*. Distribution unknown."

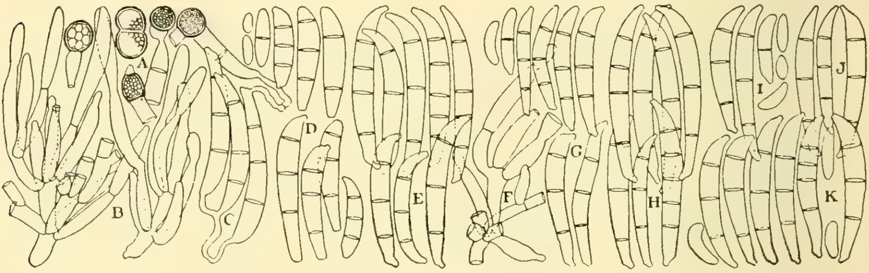

FIG. 31.—*Fusarium redolens var. Solani.* A, *Chlamydospores produced by conidia and mycelium from 120-days-old culture on potato stem plug;* B, *conidiophores from 14-days-old culture on slightly acidified hard potato agar;* C, *anastomosed conidia from 13-days-old culture on hard lima-bean agar;* D, *sporodochial conidia from 35-days-old culture on potato stem plug;* E, *pseudopionnotal conidia,* F, *conidiophores, from 14-days-old culture on slightly acidified hard potato agar;* G, *sporodochial conidia from 63-days-old culture on whole grain of rye;* H, *normal pseudopionnotal conidia from 13-days-old culture on hard lima-bean agar;* I, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar with 2 per cent glucose (the longest spore is typical);* J, *pseudopionnotal conidia from 120-days-old culture on potato stem plug;* K, *sporodochial conidia from 48-days-old culture on red raspberry cane plug*

Hab. On rotted tubers of *Solanum tuberosum*, Atlanta, New York.

Differs from *F. redolens* Wr. by somewhat narrower conidia and by color of substratum,[46] especially on potato agar rich in glucose.

Measurements of spores on different media are as follows:

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[46] Wollenweber does not directly give color of substratum of his *F. redolens*, but judging from his definite negative statement in regard to *F. conglutinans* Wr. it appears evident that *F. redolens* does not differ noticeably in color from *F. oxysporum* and the majority of other species of the section Elegans.

On slightly acidified hard potato agar, culture fourteen days old; conidia from pseudopionnotes:

Conidia: 2–septate, 0.5 per cent, 26 x 4.7μ
3–septate, 93.5 per cent, 36 x 5 (26–49 x 4.3–5.8)μ
4–septate, 6 per cent, 41.6 x 5.3 (38–46 x 4.9–6.1)μ
5– and 6–septate, rare, 43–53 x 5–6μ

On red raspberry cane plug, culture forty-eight days old; conidia from a small sporodochium:

Conidia: 0–septate, 2 per cent
1–septate, 1 per cent
3–septate, 97 per cent, 35.6 x 5 (28–45 x 4–5.9)μ

On hard lima-bean agar, culture nine days old; conidia from pseudopionnotes:

Conidia: 0–septate, 17 per cent, 10 x 3.6 (6–16 x 3–4.3)μ
1–septate, 10 per cent, 20 x 3.9 (14–28 x 3.5–4.7)μ
2–septate, 3 per cent
3–septate, 70 per cent, 34 x 4.9 (28–43 x 4.3–5.3)μ
4–septate, rare

On potato tuber plug, culture ninety-nine days old; conidia from a medium small sporodochium:

Conidia: 2–septate, rare, 27 x 4.8μ
3–septate, 100 per cent, 33.6 x 4.9 (29–39 x 4.6–5.7)μ

On potato stem plug, culture one hundred and thirteen days old:

(1) Chlamydospores, mostly unicellular
Chlamydospores: 0–septate, 6.5 x 6.1 (4.7–11 x 5.2–7.5)μ
(2) Conidia from a sporodochium
Conidia: 0– and 1–septate, very rare
3–septate, 100 per cent, 34.5 x 4.5 (24–39 x 3.9–5.1)μ
4–septate, very rare, about the same size as 3–septate

On whole steamed potato tuber, culture forty-nine days old; conidia from a medium small sporodochium:

Conidia: 0– and 1–septate, rare
3–septate, 100 per cent, 31 x 4.3 (29–36 x 4.1–4.8)μ

On rye grain, culture sixty-three days old; conidia from a small sporodochium:

(1) Conidia: 0–septate, 5 per cent
1–septate, 3 per cent
2–septate, 1 per cent
3–septate, 91 per cent, 34.6 x 4.6 (28–41 x 3.8–5.2)μ
4–septate, rare
5–septate, very rare

(2) Chlamydospores in mycelium, terminal, about the same size as those on potato stem plug

On corn agar, culture one hundred and seventy-three days old, chlamydospores intercalary and terminal:

Chlamydospores: 0–septate (in conidia), 9.4 x 8.3 (7–11 x 5.2–9)μ
1–septate (in mycelium), 15 x 9 (11–20 x 7–11)μ

On hard lima-bean agar with 2 per cent glucose, culture one hundred and eighteen days old:

(1) Chlamydospores not numerous; in conidia, 0–septate, 8.7 x 8.5μ; in mycelium, 0– and 1–septate, and also in small clusters of three

(2) Sporodochial conidia

Conidia: 1–septate, rare
3–septate, 94 per cent, 37 x 5 (28–44 x 4.6–5.9)μ
4–septate, 6 per cent, 40 x 5.05 (37–44 x 4.7–5.9)μ
5–septate, rare, about the same size as 4–septate

On hard lima-bean agar, culture five days old; chlamydospores (in conidia) not observed; conidia from pseudopionnotes:

Conidia: 0–septate, 10 per cent, 11 x 3.6μ
1–septate, 3 per cent, 22 x 4μ
2–septate, 1 per cent
3–septate, 86 per cent, 41 x 4.9 (33–53 x 4.6–5.2)μ
4–septate, rare
5–septate, very rare

On the same medium as above, culture thirteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
3–septate, 97 per cent, 41 x 4.9 (34–49 x 4.2–5.2)μ
4–septate, 3 per cent, 43 x 5 (36–48 x 4.7–5.2)μ
5–septate, rare, 45 x 5.1 (43–47 x 4.8–5.2)μ

Average of the above measurements:
Conidia: 0–septate, 3.5 per cent, 10.5 x 3.6μ
1–septate, 1.5 per cent, 21 x 3.95μ
2–septate, 0.5 per cent, 26.5 x 4.75μ
3–septate, 93 per cent, 36.4 x 4.86μ
4–septate, 1.5 per cent, 41.5 x 5.12μ
5–septate, rare, 44.3 x 5.2μ
6–septate, very rare

36. **Fusarium lutulatum** n. sp. (Figs. 1F$_1$ and 32; Pl. II, figs. 5 and 6; Pl. V, fig. 3)

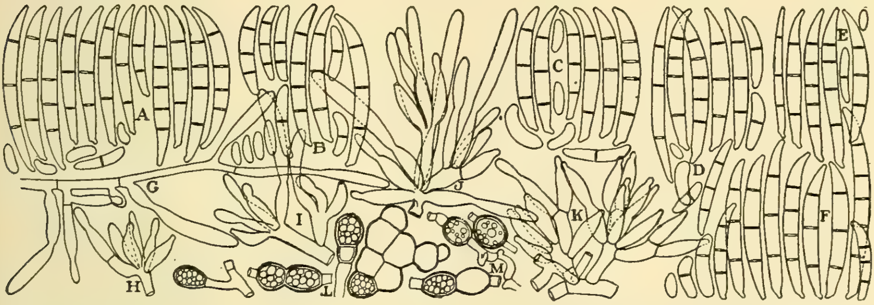

Fig. 32.—*Fusarium lutulatum.* A, *Conidia from plectenchymic sporodochium from 60-days-old culture on red raspberry cane plug;* B, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar with 2 per cent glucose;* C, *pseudopionnotal conidia from 5-days-old culture on hard lima-bean agar;* D, *sporodochial conidia from 63-days-old culture on whole grain of rye;* E, *sporodochial conidia from 49-days-old culture on whole steamed potato tuber;* F, *pseudopionnotal conidia from 23-days-old culture on slightly acidified hard potato agar;* G, H, I, J, *conidiophores from 5-days-old culture on hard lima-bean agar;* K, *conidiophores from 23-days-old culture on slightly acidified hard potato agar;* L, *terminal and intercalary chlamydospores from 63-days-old culture on whole grain of rye;* M, *chlamydospores from 25-days-old culture on corn agar*

Macroconidia gradually attenuate toward the apex, usually distinctly pedicellate and uniformly curved throughout, without stronger curvature near apex, typically 3–septate, 34 x 4 (28–38 x 3.7–4.5)μ, also 2– to 5–septate; from small to medium sporodochia (up to 2 millimeters in diameter), often converging into pseudopionnotes; aërial mycelium, when present, short, (mostly from 1 to 2 millimeters high), white, often, especially on agars in plate cultures, absent; color of conidia from nearly white (on aërial mycelium in the form of coarse powder) to dark vinaceous purple; typical variation of spore color shown in Plate II, figures 5 and 6; sometimes on potato stem plug, from one to a few large sporodochia (2 millimeters in diameter) of a bright orange color produced; substratum from colorless to that of the conidial masses; small bluish black sclerotia ($\frac{1}{2}$ millimeter in diameter) sometimes produced, and then in great numbers all over the substratum (on potato tuber plug); zonation of colony very faint or none on neutral agars in plate cultures.

Hab. On soft and dry rotted tubers of *Solanum tuberosum*, Atlanta, New York.

Latin description.—Macroconidiis gradatim in apicem attenuatis, plerumque distincte pedicellatis et aequabiliter curvatis, non ad apicem curvatioribus, typice 3–septatis, 34 x 4.23 (28–38 x 3.7–4.5)μ, vel etiam 2–5–septatis; sporodochiis minutis vel mediocribus (usque ad 2 mm. diam.), saepe in pseudopionnotes vergentibus; aerio mycelio saepe — praecique in agaribus in culturis in patellis factis — absente; conidiis ex albido (in aerio mycelio similibus magnis granis pulveris) "dark vinaceous purple" (R); vide typicam spororum coloris variationem, Tab. II, figg. 5, 6; interdum in tuberibus Solani tuberosi, vel unis vel paucis sporodochiis (2 mm. diam.) nitide "orange" (R); substrato hyalino vel eodem colore quo conidiorum moles; interdum sclerotiis minutis livido-atris ($\frac{1}{2}$ mm. diam.), tum demum eorundem magnis numeris passim in substrato in tuberibus Solani tuberosi; zonatione coloniae vel maxime indistincta vel nulla in agaribus neutralibus in culturis in patellis factis.

Hab. In mollibus vel aridis putridis tuberibus Solani tuberosi, Atlanta, New York, Amer. bor.

Measurements of spores on different media are as follows:

On slightly acidified hard potato agar, culture twenty-three days old; conidia from pseudopionnotes:

Conidia: 0–septate, 4.5 per cent, 9 x 2.7 (6–14 x 2.5–3)μ
1–septate, 1 per cent, 18 x 3.2 (13–22 x 2.6–4)μ
2–septate, 0.5 per cent, 19.5 x 3.2 (17–23 x 2.7–3.5)μ
3–septate, 89 per cent, 35.2 x 4.5 (23–38 x 3.5–4.7)μ
4–septate, 4.8 per cent, 37.8 x 4.6 (31–40 x 4.3–4.7)μ
5–septate, 0.2 per cent, 45 x 4.7 (38–50 x 4.7)μ

On red raspberry cane plug, culture sixty days old; conidia from a medium-sized sporodochium:

Conidia: 0–septate, 25 per cent
1–septate, 5 per cent
2–septate, 1 per cent
3–septate, 65 per cent, 38 x 4.2 (27–44 x 3.5–4.7)μ
4–septate, 4 per cent, 42 x 4.4 (36–49 x 3.7–4.7)μ
5–septate, rare, about the size of 4–septate conidia

On hard lima-bean agar, culture nine days old; conidia from aërial mycelium:

Conidia: 0–septate, 57 per cent, 8.5 x 2.8 (7–12 x 2.6–3.5)μ
1–septate, 16 per cent, 14.5 x 3.25 (11–21 x 2.9–3.8)μ
2–septate, 3.5 per cent
3–septate, 23 per cent, 28 x 3.9 (23–37 x 3.5–4.4)μ
4–septate, 0.5 per cent, 35 x 3.9 (30–37 x 3.5–4.4)μ

On same media, culture twenty-two days old; conidia from small sporodochia:

Conidia: 0–septate, 3 per cent
1–septate, 1 per cent
2–septate, rare
3–septate, 84 per cent, 36 x 4 (29–40 x 3.9–4.4)μ
4–septate, 10 per cent, 39 x 4.3 (33–42 x 3.9–4.7)μ
5–septate, 2 per cent, 41 x 4.2μ (only a few measured)

On hard potato agar, culture thirty-one days old:

(1) Conidia from pseudopionnotes
Conidia: 0–septate, 26 per cent, 7.4 x 2.7 (5.2–12 x 2.6–3.5)μ
1–septate, 4 per cent, 13 x 3.3μ (only three measured)
2–septate, rare, 20 x 3.7μ (only three measured)
3–septate, 54 per cent, 35 x 4.2 (20–41 x 3.4–4.8)μ
4–septate, 12 per cent, 40 x 4.4 (35–44 x 4–4.8)μ
5–septate, 4 per cent, 43 x 4.8 (41–46 x 4–4.8)μ

(2) Chlamydospores (abundant)
(a) Terminal, 0–septate, on mycelium, 8 x 6.6 (7–9 x 6–7)μ
(b) Intercalary, in mycelium
0–septate, 8.2 x 6.2 (6–9 x 4.5–8)μ
1–septate, 11 x 5.7μ (only a few measured)
(c) In conidia, 0–septate, 6.5 x 5.9μ

On potato tuber plug, culture ninety-nine days old:
(1) Conidia from a sporodochium
Conidia: 0–septate, 5 per cent, 6.2 x 2.4 (4.5–8 x 2–2.9)μ
1–septate, 1 per cent, 13 x 2.7μ (only a few measured)
2–septate, rare, 20 x 3.3μ (only a few measured)
3–septate, 92 per cent, 32 x 3.8 (20–38 x 3–4.2)μ
4–septate, rare, 37 x 4.1μ (only one measured)
5–septate, very rare, about the size of 4–septate
(2) Chlamydospores, numerous, mostly in conidia
0–septate, 6.8 x 5.1 (5.2–7.2 x 4.6–5.3)μ
1–septate, 9.6 x 5.1 (7.8–12.3 x 4.1–6)μ, the largest 8.8 x 6.1μ

On potato stem plug, culture one hundred and twelve days old:
(1) Conidia from a sporodochium
Conidia: 0–septate, 1 per cent
1–septate, rare
3–septate, 79 per cent, 36.4 x 4.1 (33–39 x 3.9–4.7)μ
4–septate, 13 per cent, 40 x 4.2 (36–42 x 3.9–4.4)μ
5–septate, 7 per cent, 40 x 4.3 (35–45 x 4–4.7)μ
6–septate, very rare, same size as 5–septate
(2) Chlamydospores, in conidia common, mostly 0–septate, 6.3 x 5.2 (5.1–8 x 4.3–6)μ

On whole steamed potato tuber, culture forty-nine days old; conidia from a sporodochium:
Conidia: 0–septate, 4 per cent
3–septate, 86 per cent, 36 x 3.7 (22–44 x 2.9–4.1)μ
4–septate, 8 per cent, 38 x 4.1 (35–43 x 3.9–4.2)μ
5–septate, 2 per cent, 40 x 4.1 (37–44 x 4.1)μ

On rye grain, culture sixty-three days old; conidia from a small sporodochium:
Conidia: 0–septate, 35 per cent
1–septate, 12 per cent
2–septate, 1 per cent
3–septate, 45 per cent, 34 x 4 (24–44 x 3.5–4.4)μ
4–septate, 5 per cent, 41 x 4.2 (36–47 x 4–4.7)μ
5–septate, 2 per cent, 45 x 4.3 (44–51 x 4–4.7)μ

On corn agar, culture one hundred and seventy-two days old; most of the conidia with chlamydospores:

(1) Pseudopionnotal conidia (only those without chlamydospores were measured)

Conidia: 3–septate, 32 x 4μ
4–septate, 35 x 4.1μ
5–septate, 37 x 4.2μ

(2) Chlamydospores in conidia very numerous, 0–septate predominant, 7.3 x 6.8 (6–10 x 5–9)μ

On hard lima-bean agar with 2 per cent glucose, culture one hundred and eighteen days old:

(1) Chlamydospores in conidia very numerous, 0–septate predominant, 7.2 x 5.7 (6–8 x 5.2–6)μ

(2) Sporodochial conidia

Conidia: 3–septate, 99 per cent, 33 x 4.2 (29–39 x 3.8–4.7)μ
4–septate, 1 per cent, 36 x 4.3 μ (only three measured)

On hard lima-bean agar, culture five days old; conidia from aërial mycelium (chlamydospores in conidia not observed):

Conidia: 0–septate, 46 per cent, about 11 x 2.6μ
1–septate, 4 per cent, about 16 x 3.1μ
2–septate, 1.5 per cent
3–septate, 48 per cent, 32 x 4 (23–41 x 3.5–4.3)μ
4–septate, 0.5 per cent, 35 x 4.1μ (only two measured)

On same medium as above, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 25 per cent
1–septate, 6 per cent
2–septate, 1 per cent
3–septate, 63 per cent, 35 x 4.2 (30–40 x 3.9–4.7)μ
4–septate, 5 per cent, 37.8 x 4.4 (33–41 x 4–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 20 per cent 8.4 x 2.6μ
1–septate, 1.5 per cent, 15 x 3.2μ
2–septate, 3.5 per cent, 20 x 3.4μ
3–septate, 69 per cent, 33.9 x 4.07μ
4–septate, 5 per cent, 39.4 x 4.23μ
5–septate, 1 per cent, 41.1 x 4.32μ

37. *Fusarium lutulatum* var. **zonatum** n. var. (Pl. I, figs. 9 and 10; Pl. V, fig. 4)

Differs from *F. lutulatum* by slightly shorter and broader microconidia; usually by the absence of chlamydospores in conidia;[47] by conidia somewhat less pointed than those in *F. lutulatum*; by commoner production of aërial mycelium; and by more or less distinct zonation of colony growth on neutral agars. Spore color begins to develop earlier but usually does not reach the density of that of *F. lutulatum*. The organism does not produce rot of potato tubers, while *F. lutulatum*, at least in most of the inoculations made, is capable of causing such rot. No sclerotia observed.

Hab. On rotted tubers of *Solanum tuberosum* together with *F. oxysporum* var. *resupinatum* and *F. sanguineum*, at Ithaca, New York.

The size of the conidia is in many instances almost identical with that in *F. lutulatum*, and in general there is scarcely any very sharp distinction between the two organisms; but there are at least slight differences in many of the important characters, so that in general there can hardly be any serious doubt that these are two distinct, though closely related, organisms.

Average of the measurements of conidia on the same media, of the same age, and the same in other ways, as those of *F. lutulatum*, is as follows:

Conidia: 0–septate, 23 per cent, 9 x 2.8μ
1–septate, 5 per cent, 16 x 3.1μ
2–septate, 2 per cent, 22 x 3.5μ
3–septate, 69 per cent, 32.77 x 4.16μ
4–septate, 1 per cent, 39.4 x 4.4μ
5–septate, very rare, 39.3 x 4.6μ

38. **Fusarium sclerotioides** n. sp. (Figs. 10$_1$ and 33; Pl. I, figs. 11 and 12; Pl. V, fig. 1)

Microconidia gradually attenuate toward and more or less pointed at the apex, pedicellate, generally somewhat more distinctly curved near apex, and broader in the middle or in the upper third of their length, typically 3–septate, 34.7 x 4.4 (30–39.5 x 4.1–4.6)μ, also 2– to 5–septate, 4– and 5–septate being of more or less common occurrence; chlamydospores observed only in mycelium (intercalary and terminal), and not very common nor numerous when compared with all the other species of section Elegans, usually only unicellular; aërial mycelium on hard agars invariably well developed, of medium height (from 2 to 4 millimeters) and density, very frequently forming macroscopically observable knots at the hyphal tips, finally, if the conditions are right, resulting in production of numerous small sporodochia; large (up to 12 millimeters in diameter), bluish black, shiny, more or less wrinkled, sclerotia are frequently produced on potato tuber plugs; the sclerotia in some cases overgrown with aërial mycelium, and then not so conspicuous; plectenchymic bodies (from 1 to 3 or more millimeters in diameter) wartlike in appearance, white or pale flesh in color, often produced in considerable number and in some cases finally bearing masses of septate conidia; conidia, however, rarely produced on the sclerotia; color of the conidial mass somewhat variable, but usually of a tint of pinkish buff; color of substratum varying from nearly colorless when young to cinnamon red, deep vinaceous, and dark vinaceous purple. (For typical color when mature see Plate I, figure 12.)

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[47] These were observed in number only once, and then when the culture was very old and much contaminated with bacteria. It was observed generally that a bacterial contamination greatly stimulates production of chlamydospores.

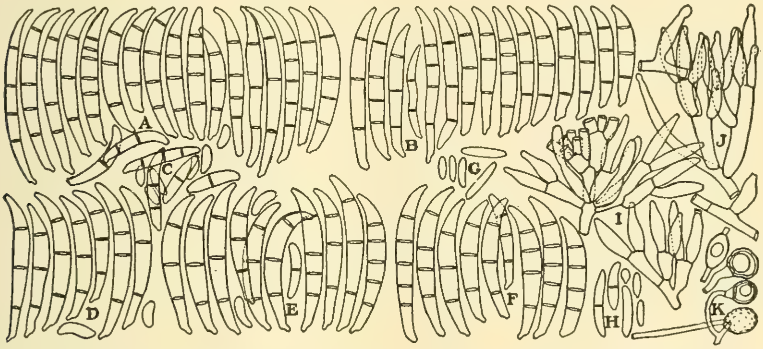

FIG. 33.—*Fusarium sclerotioides.* A, *Conidia from plectenchymic sporodochium from 14-days-old culture on potato tuber plug;* B, *conidia from plectenchymic sporodochium from 58-days-old culture on red raspberry cane plug;* C, *conidia from 24-days-old culture on slightly acidified hard potato agar;* D, *sporodochial conidia from 6-days-old culture,* E, *sporodochial conidia from 12-days-old culture,* F, *sporodochial conidia from 51-days-old culture, on hard lima-bean agar;* G, *microconidia from 13-days-old culture on hard lima-bean agar with 2 per cent glucose;* H, *microconidia from aërial mycelium,* I, *sporodochial conidiophores, from 58-days-old culture on red raspberry cane plug;* J, *conidiophore from 6-days-old culture on hard lima-bean agar;* K, *chlamydospores from 58-days-old culture on red raspberry cane plug*

Hab. On rotted tubers and in discolored fibrovascular bundles of tubers of *Solanum tuberosum* at Atlanta and Ithaca, New York, and in Louisiana.

Cultures of this organism on all media may for a long time produce microconidia almost exclusively; but if mature macroconidia from occasionally produced sporodochia are planted, macroconidia may be produced again in new cultures in great abundance and on almost any medium.

Latin description.—Microconidiis gradatim in apicem attenuatis, apice acutis, pedicellatis plerumque aliquatenus distinctius ad apicem curvatis, medio latioribus vel superiore tertio longitudinis, typice 3–septatis, 34.7 x 4.4 (30–39.5 x 4.1–4.6)μ, vel etiam 2–5–septatis, plus minusve saepe 4–5–septatis; chlamydosporis tantum in mycelio (intercalaribus et terminalibus) sed nec maxime frequentibus nec multis prae ceteris sectionis Elegantis speciebus, plerumque tantum unicellularibus; aerio mycelio in duris agaribus semper plene maturo, mediocriter alto (2–4 mm.) densoque, saepissime nodos nudo oculo conspicuos formante in hyphalibus apicibus, demum — condicionibus faventibus — multa minuta sporodochia ferente; saepe sclerotiis magnis (usque ad 12 mm. diam.) livido–atris, lucidis, plus minusve rugosis, in tuberibus Solani tuberosi; sclerotiis — si aerio mycelio obsita — minus conspicuis; saepe multis plectenchymicis corporibus (1–3 mm. diam., vel majoribus), verrucoideis, albis vel pallide carneis, vel moles septatorum conidiorum vel nullas tandem ferentibus; conidiis autem in sclerotiis raro ortis; colore conidiorum molis aliquatenus variante, sed plerumque "pinkish buff" (R) tincto; colore substrati juvenis ferme hyalino dein "cinnamon rufous" (R), "deep vinaceous" (R), "dark vinaceous purple" (R). (Vide typicam colorem maturi substrati Tab. I, fig. 12.)

Hab. In putridis tuberibus et in decoloratis fibro-vascularibus fasciculis tuberum Solani tuberosi, Atlanta et Ithaca, New York, et Louisiana, Amer. bor.

Measurements of conidia on different media are as follows:

On red raspberry cane plug, culture fifty-eight days old; conidia from a sporodochium:

Conidia: 1–septate, 1 per cent
2–septate, 1.5 per cent
3–septate, 87 per cent, 30 x 4.4 (21–40 x 3–5.1)μ
4–septate, 9 per cent, 37 x 4.5 (33–41 x 4.1–5.1)μ
5–septate, 1.5 per cent, 40 x 4.6 (35–45 x 4.3–5.1)μ

On hard lima-bean agar with 2 per cent glucose, culture thirteen days old; conidia from aërial mycelium:
Conidia: 0–septate, 90 per cent, 9.5 x 2.25 (7–15.5 x 1.75–2.4)μ
1–septate, 10 per cent, 13.4 x 2.5 (12–18 x 2.3–3)μ

On corn meal agar, culture one hundred and seventy-three days old:
Chlamydospores: 0–septate, in mycelium, 8.5 x 8.2 (7–11.5 x 5–10.5)μ

On hard lima-bean agar, culture six days old; conidia from a sporodochium:
Conidia: 0–septate, rare
1–septate, very rare
3–septate, up to 100 per cent, 37 x 4.3 (31–41 x 3.9–4.4)μ
4–septate, rare, 37 x 4.4μ (only one measured)

On same medium as above, culture fifty-one days old; conidia from a medium-sized sporodochium:
Conidia: 0–septate, 1 per cent
1–septate, 1.5 per cent
3–septate, 73 per cent, 33 x 4.5 (23–39 x 3.5–4.8)μ
4–septate, 23 per cent, 37 x 4.5 (31–42 x 4–4.8)μ
5–septate, 1.5 per cent, 38 x 4.6 (31–42 x 4.2–4.9)μ

On same medium as above, culture twelve days old; conidia from pseudopionnotes:
Conidia: 0–septate, 6 per cent
1–septate, 3 per cent
2–septate, exceptional
3–septate, 74 per cent, 35 x 4.6 [(22–) 33–39 x (3.5–) 4.1–4.8]μ
4–septate, 16 per cent, 36 x 4.7 (33–42 x 4.4–4.8)μ
5–septate, 1 per cent, 37 x 4.7 (35–40 x 4.6–4.8)μ

On potato tuber plug, culture fourteen days old; conidia from a medium-sized sporodochium:
Conidia: 0–septate, 8 per cent
1–septate, 3 per cent
2–septate, rare
3–septate, 46 per cent, 35 x 4.1 (29–43 x 3–4.7)μ
4–septate, 29 per cent, 40 x 4.2 (31–47 x 3.5–4.7)μ
5–septate, 14 per cent, 40 x 4.3 (35–47 x 4–4.7)μ

On hard oat agar, culture twenty-four days old; conidia from a sporodochium:

Conidia: 3–septate, 64 per cent, 39.5 x 4.3 (36–52 x 4–4.7)μ
4–septate, 28 per cent, 41 x 4.3 (38–45 x 4–4.7)μ
5–septate, 8 per cent, 43 x 4.5 (38–46 x 4.1–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 16 per cent, 9.5 x 2.25μ
1–septate, 3 per cent, 13.4 x 2.5μ
2–septate, rare
3–septate, 63 per cent, 34.7 x 4.4μ
4–septate, 15 per cent, 37.9 x 4.43μ
5–septate, 3 per cent, 39.35 x 4.55μ

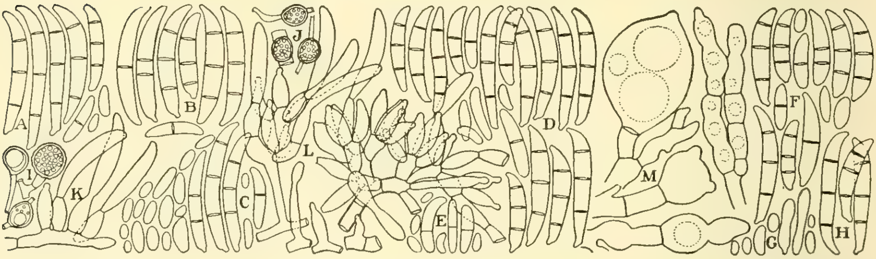

FIG. 34.—*Fusarium sclerotioides var. brevius.* A, *Pseudopionnotal conidia from 8-days-old culture on hard lima-bean agar;* B, *sporodochial conidia,* C, *conidia from aërial mycelium, from 19-days-old culture on slightly acidified hard potato agar;* D, *sporodochial conidia from 45-days-old culture on red raspberry cane plug;* E, *conidia from aërial mycelium from 13-days-old culture on hard lima-bean agar with 2 per cent glucose;* F, *sporodochial conidia,* G, *aërial conidia, from 60-days-old culture on whole steamed potato tuber;* H, *sporodochial conidia from 35-days-old culture on oats;* I, *chlamydospores from 45-days-old culture on red raspberry cane plug;* J, *chlamydospores from 25-days-old culture on corn agar;* K, L, *conidiophores from 19-days-old culture on slightly acidified hard potato agar;* M, *swollen tips of aërial hyphæ from 13-days-old culture on hard lima-bean agar with 2 per cent glucose*

39. *Fusarium sclerotioides* var. **brevius** n. var. (Fig. 34; Pl. II, figs. 1 and 2)

Differs from *F. sclerotioides* mainly by nearly constant absence of 4– and 5–septate conidia, by absence of large bluish black sclerotia, and by noticeably shorter, 3–septate conidia.

Hab. In discolored fibrovascular bundles of tubers of *Solanum tuberosum,* Alabama.

Measurements of the conidia on different media are as follows:

On red raspberry cane plug, culture forty-five days old; conidia from a plectenchymic sporodochium:

Conidia: 0–septate, 8 per cent, 15 x 3 (6–21 x 2.5–3.5)μ
1–septate, 17 per cent, 18 x 3.7 (17–23 x 3.5–4)μ
2–septate, 15 per cent, 20 x 4 (17–23 x 3.5–4.4)μ
3–septate, 60 per cent, 28.3 x 4.3 (19–39 x 3.5–4.8)μ
4–septate, rare

On slightly acidified hard potato agar, culture nineteen days old; conidia from a small sporodochium:

Conidia: 0–septate, rare
1–septate, rare
2–septate, 2 per cent, 24 x 4.2 (20–27 x 3.5–4.7)μ
3–septate, 98 per cent, 30.3 x 4.5 (21–37 x 4.1–4.8)μ

On hard lima-bean agar with 2 per cent glucose, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 98 per cent, 7.5 x 2.6 (4.3–18 x 2.3–3.7)μ
1–septate, 2 per cent, 13.5 x 3.2 (10–18 x 2.4–3.5)μ

On same medium as above, culture sixty-five days old; conidia from a sporodochium:

Conidia: 0–septate, 8 per cent, 6.4 x 2.8 (4.4–11 x 2.3–4.1)μ
1–septate, 5 per cent, 22 x 3.8 (15–29 x 3–4.3)μ
2–septate, 2 per cent, 24 x 4 (18–28 x 3.5–4.4)μ
3–septate, 85 per cent, 28 x 4.3 (24–35 x 3.7–4.8)μ

On corn meal agar, culture one hundred and seventy-three days old:

Chlamydospores: 0–septate, in conidia, 7.9 x 7 (5–11.5 x 5–9)μ
1–septate, in mycelium, 13.3 x 8.1 (11–18 x 7–9)μ

On hard lima-bean agar, culture eight days old; conidia from aërial mycelium:

Conidia: 0–septate, 55.5 per cent, 7.8 x 2.8 (5.2–13 x 2.1–3.9)μ
1–septate, 4 per cent, 13.3 x 3.2 (12–15 x 3–3.6)μ
2–septate, 0.5 per cent
3–septate, 40 per cent, 30.8 x 4.1 (23–37 x 3.9–4.2)μ

On same medium as above, culture twelve days old; conidia from pseudopionnotes:

Conidia: 0–septate, 12 per cent
1–septate, 3 per cent
2–septate, 2 per cent
3–septate, 83 per cent, 31 x 4.3 (22–41 x 4–4.7)μ
4–septate, exceptional, 41 x 4.4μ (only one measured)

On hard oat agar, culture twenty-four days old; conidia from a sporodochium:

3–septate, 100 per cent, 31.5 x 4.4 (23–35 x 3.8–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 30.5 per cent, 9.2 x 2.8μ
1–septate, 5 per cent, 16.5 x 3.5μ
2–septate, 3.5 per cent, 22 x 4μ
3–septate, 61 per cent, 29.8 x 4.3μ
4–septate, very rare, 41 x 4.4μ

40. *Fusarium oxysporum* Schlecht.[48] (Figs. 1N$_1$ and 35A; Pl. v, fig. 6)

Schlechtendal, Fl. Berol., **2**:139. 1824. Smith, E. F., and Swingle, D. B., U. S. Plant Indus. Bur., Bul. 55. 1904. Wollenweber, H. W., Phytopath. **3**:28, fig. 1F. 1913. Wollenweber, H. W., Journ. Agr. Research **2**:268. 1914.

Microconidia gradually pointed toward apex, nearly cylindrical in middle half of their length, typically not broader toward apex, usually somewhat distinctly pedicellate, 3-septate dominant, 30.4 x 4.2 (27.5–34 x 4–4.4)μ,[49] in sporodochia and pseudopionnotes; 4–septate macroconidia frequently, and 5–septate ones rarely, present; in mass usually of pinkish buff color; aërial mycelium typically well developed, of medium height (from 3 to 5 millimeters) and density, from white to (in spots on boiled rice) congo pink;

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[48] Original description of *F. oxysporum* Schlecht. (see von Schlechtendal, Flora Berolinensis **2**:139) — "F stroma convexum erumpens varium roseum superficie inaequali rugulosa, sporidiis parvis curvatis utrinque acutissimis "— is insufficient for identification of the species, and, short as it is, it sooner suggests some other species and not that of Smith and Swingle. For a detailed discussion of the matter see Appel and Wollenweber (1910: 144–146).

Wollenweber (1913 a: 28) gives his own description of *F. oxysporum* Schlecht., which, as he states (page 42 of reference cited), " includes some additions to the descriptions given by Smith and Swingle."

For the reason that, at least among pathologists and mycologists of the United States, this organism is fairly well known under this name, and after Wollenweber's description of it the meaning became definite and recognizable, the name *F. oxysporum* is retained here and is used for the organism described by Wollenweber without consideration of Schlechtendal's original description; that is, *F. oxysporum* here is as emended by Wollenweber.

[49] Wollenweber (1913 a: 28) gives the size variation as 25–45 x 3.25–4.5μ. Whether this is average or individual size variation is not stated.

substratum, on potato agar rich in glucose, vinaceous lilac, varying from colorless and orange vinaceous to pomegranate purple and vinaceous purple; plectenchymic sporodochia common on most of the media; bluish black sclerotia (up to 3 millimeters in diameter) constantly present on potato tuber plug and sometimes on different agars.

Hab. In fibrovascular bundles of diseased stems and tubers of *Solanum tuberosum*, in the United States, perhaps also in Europe, Africa, and other regions, also on *Lycopersicum esculentum* Vigna, and Pisum.

The organism was isolated alone and in association with several other Fusaria, several times from various localities in New York and in other States. The description given above is based on the study of the culture obtained through the courtesy of Dr. Wollenweber.

Measurements of the conidia are as follows:

On slightly acidified hard potato agar, culture nineteen days old; conidia from aërial mycelium close to substratum:

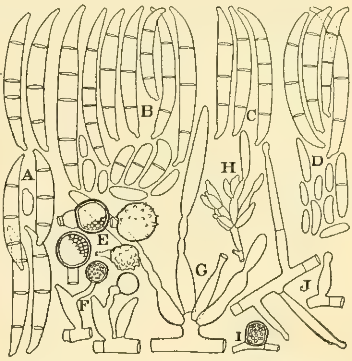

FIG. 35.— A, *Fusarium oxysporum, sporodochial conidia*

B–J, *F. oxysporum var. asclerotium:* B, *Conidia from 14-days-old culture on slightly acidified hard potato agar;* C, *conidia from 46-days-old culture on red raspberry cane plug;* D, *conidia from 7-days-old culture on hard lima-bean agar;* E, *chlamydospores from 46-days-old culture on red raspberry cane plug;* F, *terminal chlamydospores produced on normal conidiophores,* G *and* H, *conidiophores* (H *magnified 250 times*) *from 14-days-old culture on slightly acidified hard potato agar;* I, *chlamydospores from 25-days-old culture on corn agar;* J, *conidiophores from 7-days-old culture on hard lima-bean agar*

Conidia: 0–septate, 87 per cent, 8 x 2.9 (4.5–12 x 2–4) μ
1–septate, 6 per cent, 16.5 x 3.4 (11–23 x 2.5–4.1) μ
2–septate, 1 per cent, 18.5 x 3.9 (17–26 x 3–4.2) μ
3–septate, 5 per cent, 27.5 x 4 (15–39 x 3–4.7) μ
4–septate, 1 per cent, about 40 x 4.5 (36–44 x 4.3–4.8) μ

On hard lima-bean agar, culture twelve days old; conidia from aërial mycelium:

Conidia: 0–septate, 99 per cent, 6.5 x 2.6 (4.5–16 x 1.75–3.5) μ
1–septate, 1 per cent, 14 x 3.2 (10–17 x 3–3.5) μ

On same medium as above, culture sixty-five days old:
(1) Conidia from a sporodochium
Conidia: 0–septate, 3 per cent
1–septate, 1 per cent
3–septate, 93 per cent, 33.8 x 4.3 (22–42 x 4–4.7)μ
4–septate, 3 per cent, 35.7 x 4.3 (33–41 x 4.1–4.7)μ
5–septate, rare, 35 x 4.4μ (only two measured)
(2) Chlamydospores, terminal and intercalary, 0–septate dominant
Chlamydospores: 0–septate, 8.7 x 8 (7–12.5 x 7–12.5)μ
1–septate, 15.5 x 9.2 (12–22 x 7.7–14)μ
Also in short chain, 2– and 3–septate, 2–septate commoner

On hard oat agar, culture twenty-three days old; conidia from a sporodochium:
Conidia: 3–septate, 93 per cent, 30 x 4.4 (26–35 x 3.9–4.7)μ
4–septate, 6 per cent, 34.5 x 4.5 (31–39 x 4.2–4.7)μ
5–septate, 1 per cent

On corn meal agar, culture one hundred and seventy-three days old; chlamydospores abundant, terminal and intercalary:
Chlamydospores: 0–septate, in conidia, 7.2 x 6.1 (6–9 x 4.8–8)μ
0–septate, in mycelium, 8.6 x 8 (7–10 x 7–11)μ
1–septate, often observed

On hard lima-bean agar, culture fifty days old; chlamydospores intercalary and terminal, in conidia and in mycelium, the latter most commonly observed:
Chlamydospores: 0–septate dominant, 8.9 x 7.3 (5.2–11 x 5.2–11)μ

Average of the above measurements of macroconidia:
Conidia: 3–septate, 0–93 per cent, 30.4 x 4.2μ
4–septate, 0–6 per cent, 36.7 x 4.43 (34.5–60 x 4.3–4.5)μ
5–septate, 0–rare, about 35 x 4.4μ

41. *Fusarium oxysporum* Schlecht. var. **asclerotium** n. var. (Fig. 35, B to J; Pl. v, fig. 7)

Differs from *F. oxysporum* mainly by absence of sclerotia and of definite plectenchymic sporodochia; differs also in color of mycelium and in somewhat longer and narrower macroconidia.

Hab. In rotted tuber of *Solanum tuberosum*, Atlanta, New York.

The organism was isolated from a flexible, semi-soft, rotted potato tuber late in the spring. No other organisms were associated with it. Very similar organisms were isolated also from discolored fibrovascular bundles of potato tubers from various States.

Average measurements of the conidia from four different cultures are as follows:

Conidia: 0–septate, 52.5 per cent, 7.9 x 2.6μ
1–septate, 8 per cent, 15 x 3.1μ
2–septate, rare
3–septate, 39 per cent, 34 x 4 (32–36 x 3.8–4.2)μ[50]
4–septate, 0.5 per cent, 37.5 x 4.35μ
5–septate, very rare, 37.5 x 4.35μ

Chlamydospores (on corn meal culture one hundred and seventy-three days old):

0–septate, in conidia, 7.7 x 7 (6.5–11 x 5–8)μ
0–septate, in mycelium, 9 x 8 (7–11 x 5–8)μ
1–septate, in mycelium, 14 x 9.5 (11–16.5 x 7–13.4)μ[51]

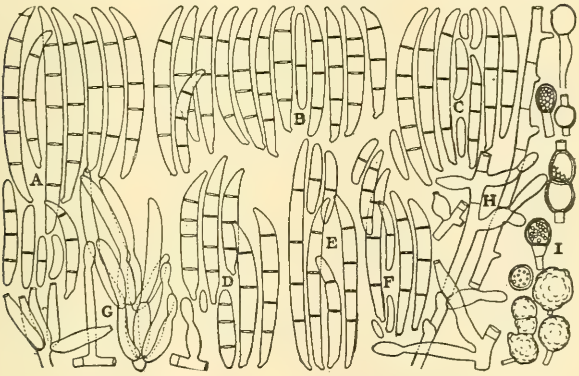

FIG. 36.—*Fusarium oxysporum var. longius.* A, *Pseudopionnotal conidia from 21-days-old culture on slightly acidified hard potato agar;* B, *conidia from 46-days-old culture on red raspberry cane plug;* C, *pseudopionnotal conidia from 5-days-old hard lima-bean agar;* D, *sporodochial conidia from 36-days-old culture on oat agar;* E, *pseudopionnotal conidia from 22-days-old culture on corn agar;* F, *conidia from 202-days-old culture on potato stem plug;* G, *conidiophores from 46-days-old culture on red raspberry cane plug;* H, *conidiophores from 5-days-old culture on hard lima-bean agar;* I, *chlamydospores from 202-days-old culture on potato stem plug*

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[50] Variation of averages of at least ten spore measurements.
[51] Variation of individual spore measurements.

42. *Fusarium oxysporum* Schlecht. var. **longius** n. var. (Figs. 1M_1 and 36)

Differs from *F. oxysporum* and other varieties of the species by longer macroconidia, typically 3–septate, 38.94 x 4.04 (34–45 x 3.6–4.4)μ, often 4– and 5–septate; no sclerotia; no macroscopical sporodochia.

Hab. In discolored fibrovascular bundles of tubers of *Solanum tuberosum* and of wilted stems, in New York, California, Maryland, and Connecticut.

On slightly acidified hard potato agar, culture twenty-one days old:

(1) Conidia from pseudopionnotes

Conidia: 0–septate, 1.5 per cent, 10 x 3 (8–12 x 2.5–3.5)μ
1–septate, 3 per cent, 18 x 3.5 (14–21 x 3–4)μ
2–septate, 0.5 per cent
3–septate, 60 per cent, 42.7 x 4.1 (28–54 x 3.5–4.8)μ
4–septate, 26 per cent, 49 x 4.3 (42–58 x 4–4.8)μ
5–septate, 9 per cent, 51.4 x 4.6 (46–60 x 4.3–4.7)μ

(2) Chlamydospores, terminal and intercalary

Chlamydospores: 0–septate, 7–9 x 5–6μ

On red raspberry cane plug, culture forty-six days old; conidia from mycelium close to substratum:

Conidia: 0–septate, 1.7 per cent, 11 x 2.6μ
1–septate, 1 per cent, 19 x 3μ
2–septate, rare
3–septate, 93 per cent, 34 x 4.4 (19–44 x 3.5–5.7)μ
4–septate, 4.3 per cent, 40 x 4.6 (35–44 x 4.2–4.8)μ

On corn meal agar, culture twenty-two days old; conidia from surface of medium:

Conidia: 0–septate, 20 per cent
1–septate, 8 per cent
2–septate, 2 per cent
3–septate, 40 per cent, 45 x 3.6 (36–54 x 3.2–4.2)μ

$$\left.\begin{array}{l}\text{4–septate, 20 per cent}\\ \text{5–septate, 10 per cent}\end{array}\right\}\text{ the largest 67 x }4\mu$$

On hard lima-bean agar with 2 per cent glucose, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 50 per cent, 8.6 x 2.5 (5.2–15 x 2.2–3)μ
1–septate, 10 per cent, 17.5 x 2.9 (12–21 x 2.3–3.5)μ
2–septate, 0.5 per cent, 21 x 3.2μ (only three measured)
3–septate, 30 per cent, 35.6 x 4 (24–50 x 3.2–4.4)μ
4–septate, 8 per cent, 45 x 4.2 (40–51 x 3.9–4.7)μ
5–septate, 1.5 per cent, 47.5 x 4.3 (42–51 x 3.9–5.1)μ

On hard lima-bean agar, culture five days old; conidia from aërial mycelium:

Conidia: 0–septate, 56 per cent, 11 x 2.4μ (only four measured)
1–septate, 4 per cent, 18 x 3.3μ (only four measured)
2–septate, 1.5 per cent
3–septate, 37 per cent, 37 x 4 (26–47 x 3.8–4.4)μ
4–septate, 1 per cent, 43 x 4.3 (40–45 x 4–4.5)μ
5–septate, 0.5 per cent, 50 x 4.4μ (only three measured)

On corn meal agar, culture one hundred and seventy-three days old:

Chlamydospores: (1) 0–septate, in conidia, 8.4 x 6.8 (7–9 x 5–8)μ
(2) 0–septate, terminal, on mycelium, 10 x 9 (9–10.5 x 7.5–10.5)μ

On potato stem plug, culture two hundred and two days old:

(1) Conidia from aërial mycelium close to substratum

Conidia: 0–septate, 40 per cent
1–septate, 20 per cent
2–septate, 10 per cent
3–septate, 30 per cent, 34.3 x 3.9 (22–42 x 3.4–4.1)μ

(2) Chlamydospores, terminal and intercalary, in spores and mycelium

Chlamydospores: 0–septate, 8.9 x 6.8 (6.2–10 x 5.2–9)μ

On hard lima-bean agar, culture twelve days old; conidia from pseudopionnotes:

Conidia: 0–septate, 2 per cent
1–septate, 1 per cent
2–septate, rare
3–septate, 70 per cent, 44 x 4.3 (35–48 x 4–4.8)μ
4–septate, 23 per cent
5–septate, 4 per cent, 46 x 4.3 (40–53 x 4.1–5.2)μ

On hard oat agar, culture fourteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, 4 per cent, 10 x 2.5μ
1–septate, 1 per cent
2–septate, rare
3–septate, 70 per cent, 44 x 4.04 (39–50 x 3.5–4.7)μ
4–septate, 20 per cent, 45 x 4.2 (41–50 x 4–4.7)μ
5–septate, 5 per cent, 49 x 4.46 (42–51 x 4.3–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 24 per cent, 10 x 2.6μ
1–septate, 7 per cent, 17.5 x 3.2μ
2–septate, 1 per cent, 21 x 3.2μ
3–septate, 52 per cent, 38.94 x 4.04μ
4–septate, 12 per cent, 44.25 x 4.35μ
5–septate, 4 per cent, 48.7 x 4.4μ

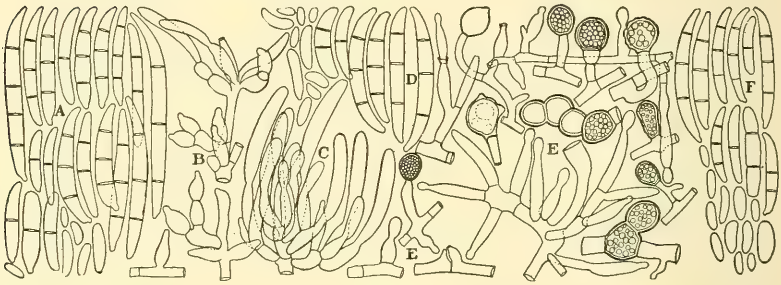

FIG. 37.—*Fusarium oxysporum var. resupinatum.* A, *Pseudopionnotal conidia from 15-days-old culture on slightly acidified hard potato agar;* B, *conidiophores from 6-days-old culture on hard lima-bean agar;* C, *conidiophore from 12-days-old culture on hard lima-bean agar;* D, *pseudopionnotal conidia from 6-days-old culture on hard lima-bean agar;* E, *conidiophores and chlamydospores,* F, *conidia, from 47-days-old culture on red raspberry cane plug*

43. *Fusarium oxysporum* Schlecht. var. **resupinatum** n. var. (Figs. 1L$_1$ and 37; Pl. I, figs. 7 and 8; Pl. V, fig. 5)

Differs from *F. oxysporum* by absence of sclerotia, from var. *asclerotium* and var. *longius* by shorter and somewhat narrower macroconidia. Mycelium on different agars typically entirely resupinate (Pl. I, figs. 7 and 8).

Hab. In discolored fibrovascular bundles of stem and tubers of *Solanum tuberosum*, United States.

This fungus and *F. oxysporum* var. *longius* are the organisms that were found very commonly present in discolored fibrovascular bundles of potato tubers and wilted plants. These organisms are also, as it seems, most commonly recognized by various pathologists as *F. oxysporum* Schlecht., as described by Smith and Swingle (1904); at least cultures of organisms undoubtedly belonging here were received under the name *F. oxysporum* from Mr. Wight in California, Dr. Taubenhaus in Delaware, and Dr. Clinton in Connecticut. The chief difference between these varieties of *F. oxysporum* of Smith and Swingle lies in the absence of sclerotia.

Measurements of spores on different media are as follows:

On slightly acidified hard potato agar, culture fifteen days old; conidia from thin pseudopionnotes:

Conidia: 0–septate, 74 per cent, 8 x 2.7 (5–15 x 2–4.3)μ
1–septate, 9 per cent, 21 x 3 (12–27 x 2.3–4.8)μ
2–septate, 4 per cent, 26 x 3 (20–37 x 2.2–4.1)μ
3–septate, 13 per cent, 35 x 4.2 (22–57 x 3.5–4.7)μ
4–septate, rare, 45 x 4.2μ

On red raspberry cane plug, culture forty-seven days old; conidia from aërial mycelium:

Conidia: 0–septate, 98 per cent, 6.5 x 2.7 (4.5–21 x 2.3–4.3)μ
1–septate, 2 per cent, 18 x 3.2 (12–24 x 2.8–4.1)μ
2–septate, rare, 22 x 3.4 (17–24 x 3–4.1)μ
3–septate, rare, 29 x 3.6 (19–36 x 3–4)μ

On hard lima-bean agar with 2 per cent glucose, culture fifteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 97 per cent, 7.1 x 2.6 (5.2–16 x 1.8–4.3)μ
1–septate, 2 per cent, 16 x 3.1 (10–18 x 2.3–3.8)μ
2–septate, 0.5 per cent
3–septate, 0.5 per cent, 28 x 3.5 (23–36 x 2.4–4)μ

On corn meal agar, culture one hundred and seventy-three days old:

Chlamydospores: 0–septate, in conidia, 7.2 x 7 (5.8–9 x 5–8)μ
0–septate, in mycelium, 9.6 x 8.3 (7–16 x 7–11.5)μ
1–septate, in mycelium, 14.5 x 9.2 (13–18 x 7–11)μ

On hard lima-bean agar, culture six days old; conidia from pseudopionnotes:

Conidia: 0–septate, 82 per cent
1–septate, 5 per cent
2–septate, 2 per cent
3–septate, 11 per cent, 28 x 4.1 (22–36 x 3.9–4.7)μ

On the same medium as above, culture twelve days old; conidia also from pseudopionnotes;

Conidia: 0–septate, 15 per cent
1–septate, 8 per cent
3–septate, 77 per cent, 33.5 x 4.1 (29–37 x 4–4.5)μ; the smallest 21 x 3.4μ, the largest 40 x 4.5μ (both 3–septate)

Chlamydospores all kinds except terminal, 0–septate dominant, measuring 8.3 x 6.7 (6–10.5 x 5.2–8)μ

Average of the above measurements:

Conidia: 0–septate, 73 per cent, 7.2 x 2.7μ
1–septate, 5 per cent, 18 x 3.1μ
2–septate, 1.5 per cent, 24 x 3.2μ
3–septate, 20.5 per cent, 30.7 x 3.9μ
4–septate, rare, 45 x 4.2μ

X. SECTION DISCOLOR Wr., Phytopath. 3:31, fig. 1, G, H, J, 1913

Conidia sickle-shaped, at the middle nearly cylindrical or broadened toward the apex, somewhat abruptly apically attenuated, distinctly pedicellate; mostly 3– to 5–septate, 5–septate dominant; microconidia typically absent; chlamydospores intercalary only, usually scant; mycelium typically well developed, with from nearly white to orange color as type. Substratum from nearly colorless to chamois, pomegranate purple, and spectrum red. Color of conidia very variable, mostly cinnamon to orange.

Differs from sections Elegans and Martiella by absence of microconidia, by the conidia being typically somewhat abruptly attenuate, by absence of terminal chlamydospores, and by absence of vinaceous, drab-gray, tawny-olive, and blue color of substratum. By the shape of conidia this section occupies an intermediate position between sections Elegans and Martiella; by its color it is closely related to sections Roseum and Ferruginosum.

44. *Fusarium trichothecioides* Wr. (Figs. 1, U₁ and V₁, and 38; Pl. IV, fig. 8)

Jamieson, C. O., and Wollenweber, H. W., Journ. Washington Acad. Sci. **2**:146–152, fig. 1. 1912.

Syn. *Fusarium tuberivorum* Wilcox and Link, Nebraska Agr. Exp. Sta., Research bul. 1:48. 1913.

Conidia of comma and discolor types the former predominating and under ordinary cultural conditions occurring almost exclusively, mostly 1–septate, 16 x 4.6 (14–17 x 4.2–5.4)μ, often 0– to 3–septate, seldom 4– or 5–septate, 6–septate rare; sporodochial conidia sickle-shaped, 3– to 5–septate, 24–42 x 4.5–5.5μ.[52] Typical conidiophores of *comma* stage shown in figure 38. Sporodochial conidiophores similar to those of *F. discolor*. *F. trichothecioides* can be recognized at once by color and appearance of its powdery masses of spores produced on aërial mycelium (Pl. IV, fig. 8). Chlamydospores few and not prominent.

Hab. On rotted tubers of *Solanum tuberosum*, United States.

This species is noticeably different from all other species of the section Discolor. It is listed under this section chiefly because of its resemblance in the sporodochial form. Perhaps it should be made the type of a new section.

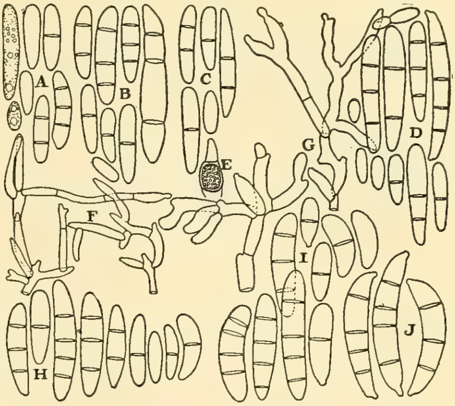

FIG. 38.—*Fusarium trichothecioides.* A, *Conidia from 41-days-old culture on rye straw;* B, *conidia from 70-days-old culture on potato stem plug;* C, *conidia from 73-days-old culture on red raspberry cane plug;* D, *conidia from 15-days-old culture on slightly acidified hard potato agar;* E, *chlamydospore in conidium from 73-days-old culture on red raspberry cane plug;* F, *conidiophores (magnified 250 times) from 70-days-old culture on potato stem plug;* G, *conidiophores from 15-days-old culture on slightly acidified hard potato agar;* H, *conidia from 5-days-old culture on hard lima-bean agar;* I, *comma,* J, *discolor, type of conidia from 5-days-old culture on potato tuber plug*

The organism, under the name *F. tuberivorum* Wilcox and Link, was extensively studied by Wilcox, Link, and Pool (1913). The identity of *F. tuberivorum* and *F. trichothecioides* Wr. is discussed by Wollenweber (1913 c:206). In the writer's opinion there is no doubt that this is correct.

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[52] The size of 3– to 5–septate conidia is as given by Wollenweber. The writer isolated this Fusarium from specimens of rotted potato tubers received from North Dakota in July, 1912, and from Texas in December, 1913. No special study of this organism was made at first, because it is easily recognizable even after a superficial examination, and also because its description as given by Wollenweber (see Jamieson and Wollenweber, 1912) is entirely sufficient for its identification.

The few measurements of the conidia on different media are as follows:

On slightly acidified hard potato agar, culture fifteen days old:

Conidia: 0–septate, 23 per cent, 8.2 x 3.8 (5–15 x 3–4.7)μ
1–septate, 59 per cent, 19 x 4.2 (12–26 x 3.5–4.7)μ
2–septate, 8 per cent, 22.4 x 4.3 (16–35 x 4.3–5.2)μ
3–septate, 10 per cent, 28.1 x 5 (20–39 x 4.3–5.3)μ
4– and 5–septate, few, 40–45 x 5μ

On red raspberry cane plug, culture seventy-three days old:

Conidia: 0–septate, 39 per cent, 12 x 4.2 (7–14 x 3–4.8)μ
1–septate, 50 per cent, 14 x 4.3 (14–20 x 3.9–4.8)μ
2–septate, 11 per cent, 22 x 4.5 (19–32 x 4.1–5.2)μ

On potato tuber plug, culture five days old, kept at a low (from 3° to 7° C.) temperature:

Conidia: 0–septate, 6 per cent, 14 x 5.2 (10–17 x 4.8–5.5)μ
1–septate, 53 per cent, 16 x 5.4 (12–23 x 4.4–7)μ
2–septate, 15 per cent
3–septate, 19 per cent, 26 x 5.8 (19–42 x 5.2–7)μ
4–septate, 6 per cent, 35 x 6.2 (33–40 x 5.4–7)μ
5–septate, 1 per cent, 38 x 6.3 (31–52 x 5.4–7)μ
6–septate, exceptional, 41 x 6.3 (39–47 x 6–7)μ (only three measured)

Average of the above measurements:

Conidia: 0–septate, 23 per cent, 11 x 4.4μ
1–septate, 54 per cent, 16 x 4.6μ
2–septate, 11 per cent, 21 x 4.8μ
3–septate, 10 per cent, 27 x 5.4μ
4–septate, 2 per cent
5–septate, rare, 38 x 6.3μ
6–septate, very rare

45. **Fusarium subpallidum** n. sp. (Fig. 39; Pl. v, fig. 12)

Conidia sickle-shaped, typically abruptly constricted at apex, slightly pedicellate to papillate, somewhat broader in the middle, mostly 5–septate, 29.1 x 5.53 (28–32.5 x 5.4–5.8)μ, 3– and 4–septate common, 6– and 7–septate very rare; chlamydospores common, mostly in long chains; aërial mycelium well developed; plectenchymic sporodochia (up to 3 millimeters in diameter) common; color of aërial mycelium from white to sea-foam yellow and honey yellow; color of substratum, on agars rich in glucose, mostly from chamois to raw sienna and antique brown in some old cultures; color of conidia, in mass, commonly from pinkish buff to pale orange, sometimes from green to blue.[53]

Hab. On superficial dry rot of tubers of *Solanum tuberosum.* The organism was isolated from rotted potato tubers received from Edgerton, Louisiana.

Latin description.—Conidiis falciformibus, typice subito apice constrictis, parum pedicellatis demum papillatis, medio aliquatenus latioribus, plerumque 5–septatis, 29.1 x 5.53 (28–32.5 x 5.4–5.8)μ, saepe etiam conidiis 3–4–septatis; conidiis 6–7–septatis rarissimis; chlamydosporis frequentibus plerumque longis catenulatis; aerio mycelio plene maturo; plectenchymicis sporodochiis (0–3 mm. diam.) frequentibus; aerio mycelio ex albo "sea-foam yellow" (R) vel "honey-yellow" (R); substrato, in agaribus perglucosis, plerumque e "chamois" (R) "raw sienna" (R) vel etiam in nonnullis culturis maturis "antique brown" (R); conidiis in totum plerumque e "pinkish buff" (R) pallide "orange" (R) vel interdum e viridi caeruleis.

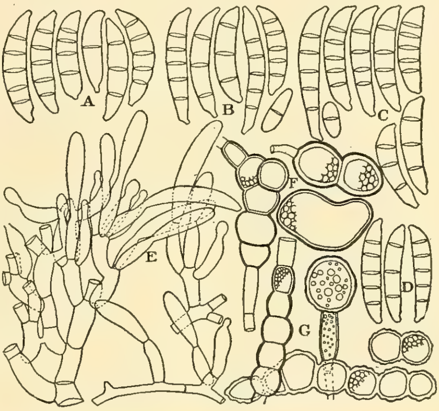

FIG. 39.—*Fusarium subpallidum.* A, *Sporodochial conidia from 70-days-old culture on hard potato agar;* B, *sporodochial conidia from 35-days-old culture on hard oat agar;* C, *conidia from a thin slimy layer from 15-days-old culture on slightly acidified hard potato agar;* D, *conidia from aërial mycelium from 52-days-old culture on red raspberry cane plug;* E, *conidiophores from 15-days-old culture on slightly acidified hard potato agar;* F, *chlamydospores from 175-days-old culture on corn agar;* G, *conidiophores from 52-days-old culture on red raspberry cane plug*

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[53] A very exceptional color for an organism of the section Discolor, and observed only in *F. subpallidum.* When the conidia are from green to blue in mass, some of them appear under the microscope very densely blue as if stained with methyl blue. The same was observed also in the case of *F. coeruleum, F. Martii,* and *F. Solani* var. *cyanum.*

Hab. In tuberibus aridis et extra putridis Solani tuberosi. Fungus e tuberibus Solani tuberosi putridis ab Edgerton in Louisiana, Amer. bor. receptis, sejungebatur.

Measurements of conidia on various media are as follows:

On slightly acidified hard potato agar, culture fifteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, rare
2–septate, rare
3–septate, 23 per cent, 24.4 x 5.2 (16–30 x 4.7–5.7)μ
4–septate, 31 per cent, 25.6 x 5.5 (19–32 x 5.2–5.9)μ
5–septate, 45 per cent, 28 x 5.7 (24–34 x 4.7–5.9)μ
6– and 7–septate, 1 per cent, 31 x 6.1 (26–35 x 5.7–6.4)μ

On red raspberry cane plug, culture fifty-nine days old; conidia from aërial mycelium:

Conidia: 3–septate, 40 per cent, 24 x 4.7 (20–28 x 4.3–5)μ
4–septate, 18 per cent
5–septate, 42 per cent, 28 x 5.2 (24–31 x 5–5.3)μ (only four measured)

On hard potato agar, culture one hundred and seventy-five days old:

(1) Conidia from a sporodochium

Conidia: 3–septate, 4 per cent, 26 x 5.3 (23–28 x 5.2–5.4)μ
4–septate, 1 per cent
5–septate, 95 per cent, 29 x 5.8 (26–33 x 5.2–6.1)μ

(2) Chlamydospores

0–septate, in conidia, 8 x 7.5 (7–9 x 6–9)μ
1–septate, in mycelium, 15.5 x 10.3 (10–18 x 8.7–13.2)μ, the largest observed being 21 x 14μ

On medium soft potato agar, culture fifty days old:

(1) Conidia from thin pseudopionnotes

Conidia: 3–septate, 28 per cent, 25 x 5.4 (26–29 x 4.8—5.9)μ
4–septate, 23 per cent, about the size of 5–septate
5–septate, 49 per cent, 28 x 5.6 (24–32 x 5.2–6.3)μ

(2) Chlamydospores

0– and 1–septate, in clusters and chains, 7–16μ in diameter

On hard oat agar, culture twenty-three days old; conidia from sporodochium:

Conidia: 5–septate, 100 per cent, 32.5 x 5.4 (24–35 x 5.2–5.7)μ

Average of the above measurements:

Conidia: 0– to 2–septate, rare
3–septate, 19 per cent, 24.5 x 5.2μ
4–septate, 15 per cent, 25.6 x 5.5μ
5–septate, 66 per cent, 29.1 x 5.53μ
6– and 7–septate, very rare, 31 x 6.1μ

46. *Fusarium subpallidum* var. **roseum** n. var. (Fig. 1, S_1 and T_1; Pl. IV, fig. 3)

Differs from *F. subpallidum* by lower septation of conidia, the majority being 3–septate, 25.25 x 4.9 (22.5–27 x 4.7–5)μ, and by a tint or shade of from Tyrian rose to pomegranate purple in substratum on agars rich in glucose.

Hab. On rotted tubers of *Solanum tuberosum*, Kentucky.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture fifteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, few
1–septate, 1 per cent, 17.5 x 4.7 (10–18 x 4.7)μ
2–septate, 1 per cent
3–septate, 44 per cent, 24.5 x 4.8 (18–29 x 4.7–5.3)μ
4–septate, 35 per cent, 27 x 4.9 (24–30 x 4.7–5.3)μ
5–septate, 19 per cent, 29 x 5.3 (24–32 x 4.7–5.7)μ

On red raspberry cane plug, culture seventy-three days old; conidia from a sporodochium:

Conidia: 0–septate, exceptional
1–septate } 1 per cent { 12 x 4.1 (10–14 x 3.8–4.4)μ
2–septate } { 23 x 4.8μ (only a few measured)
3–septate, 34 per cent, 26 x 5 (21–28 x 4.7–5.3)μ
4–septate, 35 per cent, 28 x 5.3 (23–30 x 4.8–5.7)μ
5–septate, 30 per cent, 30 x 5.3 (24–33 x 5–5.7)μ

On hard lima-bean agar, culture thirty-two days old; conidia from a sporodochium:

Conidia: 1–septate, 16 per cent
2–septate, 7 per cent
3–septate, 77 per cent, 22.5 x 4.8 (18–25 x 4.7–5.3)μ
4–septate, few, 26 x 5.1 (25–30 x 4.7–5.3)μ

On hard oat agar, culture twenty-three days old; conidia from a sporodochium:

Conidia: 3–septate, 90 per cent, 27 x 4.7 (24–34 x 4.1–4.9)μ
4–septate, 9 per cent, 29 x 4.9 (24–34 x 4.1–5.2)μ
5–septate, 1 per cent, 33 x 4.9 (30–40 x 4.8–5.2)μ

Average of the above measurements:

Conidia: 0–septate, rare
1–septate, 4.5 per cent, 17.5 x 4.7μ
2–septate, 2 per cent
3–septate, 61 per cent, 25.25 x 4.9μ
4–septate, 20 per cent, 27.5 x 5.1μ
5–septate, 12.5 per cent, 29.5 x 5.3μ

47. **Fusarium clavatum** n. sp. (Figs. 1R_1 and 40; Pl. III, figs. 11 and 12; Pl. VII, fig. 2)

Conidia sickle-shaped, typically distinctly broader in upper third of their length, somewhat suddenly constricted at the apex, slightly pedicellate, mostly 3– to 5–septate, 5–septate measuring 36.2 x 5.05 (32–46 x 4.8–5.2)μ; aërial mycelium of a medium development (2 to 4 millimeters high, more or less loose) to nearly absent, and then substratum covered with pseudopionnotes; chlamydospores scant, not in long chains; color of aërial mycelium and substratum from white to light pink and that shown on Plate III, figures 11 and 12; color of pseudopionnotes from pale pink to deep olive-buff and chocolate brown (Pl. III, fig. 11); color of sporodochia bright orange (Pl. VII, fig. 2).

Hab. On rotted tubers of *Solanum tuberosum,* together with *F. coeruleum,* Castile, New York.

Latin description.—Conidiis falciformibus, typice distincte latioribus in superiore tertio longitudinis, apice aliquatenus subito constrictis, parum pedicellatis, plerumque 3–5–septatis, 5–septatis, 36.2 x 5.05 (32–46 x 4.8–5. 2)μ; aerio mycelio mediocriter maturo (2–4 mm. alt., plus minusve laxo) vel ferme nullo, quae cum ita sint substrato pseudopionnotibus obsito; chlamydosporis paucis, non catenulatis; aerio mycelio substratoque ex albo pallide rubello vel eodem colore quod in Tab. III, figg. 11, 12, exhibente; pseudopionnotibus e pallide rubello "olive-buff" (R) et "chocolate brown" (R); sporodochiis nitide "orange" (R).

Hab. In tuberibus putridis Solani tuberosi una cum F. coeruleo, Castile, New York, Amer. bor.

Measurements of conidia on various media are as follows:

On red raspberry cane plug, culture ninety-two days old; conidia from pseudopionnotes:

Conidia: 3–septate, 5 per cent, 27 x 4.2 (20–35 x 4–4.7)μ
5–septate, 95 per cent, 35 x 4.8 (29–39 x 4.3–5.7)μ

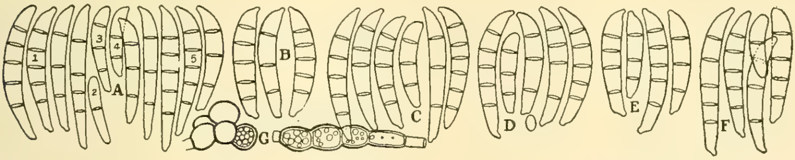

Fig. 40.—*Fusarium clavatum.* A, *Conidia from a thin layer near substratum from 92-days-old culture on red raspberry cane plug (conidia 1 and 5 are typical, but in certain areas conidia like 2, 3, and 4 were present almost to the exclusion of the other kinds);* B, *conidia from thin layer from 120-days-old culture on potato stem plug;* C, *pseudopionnotal conidia from 41-days-old culture on rye straw;* D, *pseudopionnotal conidia from 32-days-old culture on hard oat agar;* E, *pseudopionnotal conidia from 10-days-old culture on potato agar;* F, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar;* G, *chlamydospores from 10-days-old culture on potato agar*

On hard lima-bean agar with 2 per cent glucose, culture twenty-two days old; conidia from aërial mycelium:

Conidia: 3–septate, 60 per cent, 26 x 4.9 (19–38 x 4.3–5.3)μ
4–septate, 16 per cent, 32 x 5.2μ (only one measured)
5–septate, 24 per cent, 32 x 5.15 (29–36 x 4.7–5.3)μ

On medium soft potato agar, culture ten days old; conidia from aërial mycelium (chlamydospores intercalary, in chains and clusters):

Conidia: 1–septate, 1 per cent, 16 x 4.4μ (only three measured)
3–septate, 82 per cent, 26 x 5 (23–31 x 4.7–5.4)μ
4–septate, 9 per cent, 29 x 5.1 (28–32 x 4.7–5.3)μ
5–septate, 8 per cent, 32 x 5.2 (29–34 x 4.7–5.3)μ

On hard lima-bean agar, culture nine days old; conidia from aërial mycelium close to substratum:

Conidia: 1–septate, very rare
2–septate, very rare
3–septate, 33 per cent, 30 x 4.6 (19–34 x 4.3–4.8)μ
4–septate, 23 per cent
5–septate, 44 per cent, 36 x 5 (33–40 x 4.6–5.4)μ

On same medium as above, culture fifteen days old; conidia from pseudopionnotes:

Conidia: 2–septate, very rare
3–septate, 36 per cent, 33 x 5 (22–39 x 4.6–5.3)μ
4–septate, 22 per cent
5–septate, 42 per cent, 36.5 x 5.1 (33–41 x 4.9–5.3)μ

Average of the above measurements:

Conidia: 1–septate, rare, 16 x 4.4μ
2–septate, very rare
3–septate, 43 per cent, 28.5 x 4.75μ
4–septate, 15 per cent
5–septate, 42 per cent, 36.2 x 5.05μ

48. *Fusarium discolor* Ap. et Wr. (Fig. 1Q_1 and 41; Pl. IV, fig. 4; Pl. V, fig. 11)

Appel, O. and Wollenweber, H. W., Arb. K. biol. Anst. Land– u. Forstw. **8**:108–115; Pl. I, figs. 50 to 59; Pl. III, fig. 7. 1910.

Conidia for greater part of their length of nearly even diameter, sickle-shaped, gradually attenuated, often somewhat suddenly constricted at the apex, pedicellate, mostly 3– to 5–septate, 5–septate measuring 38.7 x 5.1 (35–40 x 4.6–5.1)μ,[54] on aërial mycelium, in pseudopionnotes, and in plectenchymic sporodochia; chlamydospores scant, not in long chains; aërial mycelium from poorly to well developed, from pale pink-buff to ochraceous orange and Eugenia red; color of substratum, on agars rich in glucose, from pale salmon at an early stage and warm sepia in old cultures to tyrian and ox blood; color of conidia mostly from light ochraceous salmon to ochraceous buff.

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[54] Size of 5–septate conidia according to Wollenweber's data (see Appel and Wollenweber 1910 : 111–112) is 36 x 5.1 (29–39 x 4.5–5.5)μ. The writer's measurements were taken from cultures of the same organism. Average size of *F. discolor* strain isolated in United States is, for 3–septate conidia, 30.2 x 4.4μ (from 25 to 98 per cent), and for 5–septate conidia, 38.4 x 4.85 (33.5–44 x 4.5–5)μ (from 0 to 53 per cent).

Hab. In stems and rotten tubers of *Solanum tuberosum*, in Germany and in the United States.

Differs from *F. clavatum* mainly by indistinctly clavate conidia and by presence of denser hues of red color in substratum.

Measurements of conidia on various media are as follows:

On slightly acidified hard potato agar, culture twenty-four days old; conidia from pseudopionnotes:

Conidia: 1–septate, rare
3–septate, 11 per cent, 32.3 x 4.3 (27–37 x 4.1–4.9)μ
4–septate, 14 per cent, 36 x 4.5 (32–42 x 4.3–5)μ
5–septate, 75 per cent, 39.2 x 4.7 (31–46 x 4.3–5.7)μ
6–septate, few, 53 x 5.8μ (only one measured)

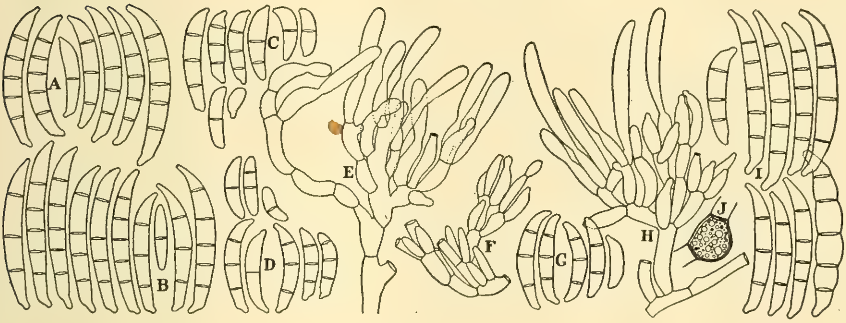

Fig. 41.—*Fusarium discolor.* A, *Conidia from a small plectenchymic sporodochium;* B, *conidia from pseudopionnotes from 15-days-old culture on slightly acidified hard potato agar;* C, *conidia from 81-days-old culture on red raspberry cane plug;* D, *conidia from 36-days-old culture on potato stem plug;* E, *conidiophores from aërial mycelium from 81-days-old culture on red raspberry cane plug;* F, *conidiophores from minute sporodochia from 36-days-old culture on potato stem plug;* G, *conidia from myceliol growth from 41-days-old culture on rye straw;* H, *conidiophore from pseudopionnotes from 15-days-old culture on slightly acidified hard potato agar;* I, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar;* J, *intercalary chlamydospore from culture on corn agar*

On red raspberry cane plug, culture seventy-two days old; conidia from minute sporodochia on aërial mycelium:

Conidia: 1– and 2–septate, rare
3–septate, 27 per cent, 30 x 4.4 (19–49 x 3.7–4.8)μ
4–septate, 24 per cent, 36 x 4.6 (26–49 x 3.9–5.9)μ
5–septate, 49 per cent, 40 x 5.2 (32–50 x 4.9–5.9)μ

On potato tuber plug, culture ninety-nine days old; conidia from a sporodochium:

Conidia: 3–septate, 6 per cent, 26 x 4.7 (22–30 x 4.3–4.8)μ
4–septate, 25 per cent, 33 x 5 (28–40 x 4.6–5)μ
5–septate, 69 per cent, 35 x 5.2 (28–42 x 4.7–5.7)μ

On hard lima-bean agar with 2 per cent glucose, culture fifty-four days old; conidia from a small sporodochium:

Conidia: 1– to 3–septate, rare
4–septate, 2 per cent, 36 x 4.9μ (only two measured)
5–septate, 98 per cent, 40 x 5.2 (33–44 x 4.6–5.5)μ

On hard lima-bean agar, culture thirty-four days old; conidia from pseudopionnotes:

Conidia: 1–septate, very rare
3–septate, 49 per cent, 33.5 x 4.8 (24–44 x 4.1–6)μ
4–septate, 31 per cent
5–septate, 20 per cent, 39.3 x 5.1 (31–47 x 4.6–6.2)μ

Average of the above measurements:

Conidia: 1–septate, very rare
2–septate, very rare
3–septate, 19 per cent, 30.5 x 4.55μ
4–septate, 19 per cent
5–septate, 62 per cent, 38.7 x 5.1μ
6–septate, very rare, 53 x 5.8μ

49. *Fusarium discolor* Ap. et Wr. var. *sulphureum* (Schlecht.) Ap. et Wr. (Pl. IV, fig. 11)

Cf. Schlechtendal, Fl. Berol. **2**:134. 1824. Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:115–118. 1910.

Differs from *F. discolor* by absence of red color in mycelium and substratum, and by entirely exposed pseudopionnotes on various agars, a characteristic culture of which is shown in Plate IV, figure 11.

Average percentage of different septation types and sizes of conidia on three different media are as follows:

Conidia: 1–septate, about 0.5 per cent
2–septate, very rare
3–septate, about 25 per cent, 28.5 x 4.2μ

4–septate, about 28 per cent
5–septate, about 46.5 per cent, 40 x 4.9 (38–41 x 4.8–4.9)μ
6–septate, rare, 48 x 5.2μ

50. *Fusarium discolor* Ap. et Wr. var. **triseptatum** n. var. (Figs. 1w_1 and 42; Pl. IV, figs. 5 and 6; Pl. V, fig. 10)

Differs from *F. discolor* by dominance of 3–septate conidia 24.2 x 4.7 (22–26 x 4.5–4.9)μ, by presence of very large (up to 1.2 centimeters in diameter), warty, plectenchymic bodies (producing conidia or remaining sterile) of a pale pinkish buff with spots of a darker color, by more intense color of mycelium and substratum (see Plate IV, figures 5 and 6), and by production of larger sporodochia with darker spore masses, much the same as shown for *F. culmorum* var. *leteius* in Plate IV, figure 10.

Hab. On rotted tubers of *Solanum tuberosum* together with *F. coeruleum*, Long Island, New York.

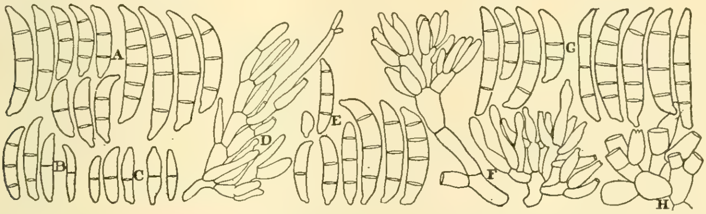

FIG. 42.—*Fusarium discolor var. triseptatum.* A, *Conidia from minute aërial sporodochia from 22-days-old culture on slightly acidified hard potato agar;* B, *conidia from 66-days-old culture on potato stem plug;* C, *conidia from aërial mycelium from culture on rye straw;* D, *conidiophore from aërial mycelium from 66-days-old culture on potato stem plug;* E, *conidia from plectenchymic sporodochia,* F, *conidiophores, from 22-days-old slightly acidified hard potato agar;* G, *conidia from plectenchymic sporodochia from 9-days-old culture on hard lima-bean agar;* H, *basal part of aërial compound conidiophore from 73-days-old culture on red raspberry cane plug*

Measurements of conidia on a few different media are as follows:

On slightly acidified hard potato agar, culture twenty-two days old; conidia from minute sporodochia on aërial mycelium:

Conidia: 0–septate, rare
1–septate, 1.6 per cent, 16 x 3.9μ
2–septate, 6.4 per cent, 18.5 x 4.3 (17–21 x 3.9–4.7)μ
3–septate, 91.2 per cent, 22.5 x 4.5 (17–39 x 3.9–5.7)μ
4–septate, 0.8 per cent
5–septate, very rare, 31 x 5.3 (28–35 x 5.2–5.8)μ

On red raspberry cane plug, culture seventy-three days old; conidia from a sporodochium:

Conidia: 0–septate, very rare
1–septate, 2 per cent, 15 x 3.7 (10–20 x 3–4.2)μ
2–septate, 8 per cent, 20 x 4.1 (17–23 x 3.6–4.7)μ
3–septate, 88 per cent, 24 x 4.6 (17–29 x 3.9–5.3)μ
4–septate, 2 per cent, 28 x 5.2 (25–30 x 4.8–5.7)μ

On hard lima-bean agar, culture nine days old; conidia from a young plectenchymic sporodochium:

Conidia: 1–septate, 2 per cent
2–septate, 3 per cent, 20 x 4.5 (13–25 x 4.1–4.7)μ
3–septate, 88 per cent, 26 x 4.9 (18–33 x 4.2–6)μ
4–septate, 6 per cent
5–septate, 1 per cent, 30 x 5.8μ (only a few measured)

Average of the above measurements:

Conidia: 0–septate, very rare
1–septate, 2 per cent, 15.5 x 3.8μ
2–septate, 6 per cent, 19.5 x 4.3μ
3–septate, 89 per cent, 24.2 x 4.7μ
4–septate, 3 per cent, 29.5 x 5.25μ
5–septate, few, 30.5 x 5.55μ

The organism seems to be so different from *F. discolor* that one may wonder why it is considered as a variety rather than an independent species, especially in view of the fact that there are some organisms designated as species which apparently differ very slightly from the other related species. The explanation lies mainly in the fact that, notwithstanding a seemingly great difference, this difference is in unstable characters — greater proportion of one type of septation instead of another, denser color, larger sporodochia, and so forth — and, moreover, in certain instances the two organisms approach each other so closely as to be distinguished only with considerable difficulty if at all.

51. *Fusarium culmorum* (W. Smith) Sacc. (Pl. IV, fig. 9; Pl. V, fig. 8)

Cf. Wollenweber, H. W., Journ. Agr. Research **2**:260–261, Pl. XVI, fig. J. 1914.

Syn. *Fusarium culmorum* W. G. Smith, Dis. Field and Gard. Crops, pp. 208–210, fig. 92. 1884.
Fusarium Schribauxi Delacr., Bul. Soc. Mycol. France **6**:99, pl. 15, fig. 1. 1890. Saccardo, Syll. Fung. **10**:726. 1890.
Fusarium culmorum (W. Sm.) Sacc., Syll. Fung. **11**:651. 1895.
Fusarium corallinum Mattirolo (non Sacc.), Mem. R. Accad. Sci. Ist. Bologna, ser. 5:**6**:677, figs. 16 and 17. 1897.
Fusarium rubiginosum Ap. et Wr., Arb. K. biol. Anst. Land- u. Forstw. 8:95–108; text fig. 8; Pl. I, figs. 31 to 48. 1910.

Conidia for a greater part of their length of an even diameter, mostly 5–septate, 38.5 x 5.85 (37–40 x 5.3–6.2)μ, somewhat suddenly constricted at apex; pedicellate, of distinctly ochraceous orange color under microscope; chlamydospores of more or less common occurrence in mycelium and in conidia, not in long chains; aërial mycelium well developed, high (up to 1 centimeter and more), very loose, at first from white to pinkish cinnamon, and then to jasper and Eugenia red; substratum, on potato agar rich in glucose, of from spectrum red to carmine-pomegranate purple, with more or less brick red color; color of conidia in mass from cinnamon and light ochraceous to mikado brown and warm sepia; sporodochia minute, separate or converging into pseudopionnotes.

Hab. On cereals and on potato tubers and some other hosts, in Europe and the United States.

The organism was isolated by the writer from rotted potato tubers, alone and in association with other Fusaria.

Measurements of conidia on different media are as follows:[55]

On slightly acidified hard potato agar, culture twenty-three days old; conidia from pseudopionnotes:

Conidia: 3–septate, 5 per cent, 33 x 5.9 (21–36 x 4.7–6.1)μ
4–septate, 10 per cent, 35 x 6.1 (30–37 x 5.7–6.4)μ
5–septate, 85 per cent, 37 x 6.2 (32–46 x 5.2–6.5)μ

On red raspberry cane plug, culture seventy-two days old:

(1) Conidia from a sporodochium

Conidia: 3–septate, 3 per cent
4–septate, 10 per cent
5–septate, 87 per cent, 40 x 6.2 (33–53 x 5.8–7)μ

---

[55] Average of the measurements given by Appel and Wollenweber (1910:106) is as follows:
Conidia: 0– to 2–septate, about 1 per cent
3–septate, about 11 per cent, 26 x 5.5μ
4–septate, about 13 per cent, 27 x 5.7μ
5–septate, about 74 per cent, 39 x 6.1μ
6–septate, about 1 per cent, 45 x 6μ

(2) Conidia from aërial mycelium

Conidia: 3–septate, 60 per cent, 30 x 4.7 (19–39 x 4.1–5.9)μ
4–septate, 25 per cent, 37 x 5 (31–42 x 4.4–5.9)μ
5–septate, 15 per cent, 39 x 5.3 (35–43 x 5.2–5.9)μ

On hard lima-bean agar with 2 per cent glucose, culture one hundred and fifty-four days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare, 8 x 4μ (only a few measured)
1–septate, rare, 12 x 3.7μ (only a few measured)
2–septate, rare
3–septate, 3 per cent, 26 x 4.7 (17–34 x 4.1–5.5)μ
4–septate, 7 per cent
5–septate, 89 per cent, 38 x 5.7 (33–47 x 5.2–6.1)μ
6–septate, 1 per cent, 49 x 5.7μ (only a few measured)
7–septate, very rare, 56 x 6.1μ (only a few measured)

Average of the above measurements:

Conidia: 0–septate, very rare, 8 x 4μ
1–septate, very rare, 12 x 37μ
2–septate, very rare
3–septate, 18 per cent, 29.67 x 5.1μ
4–septate, 13 per cent
5–septate, 69 per cent, 38.5 x 5.85μ
6–septate, few, 49 x 5.7μ
7–septate, exceptional, 56 x 6.1μ

52. *Fusarium culmorum* (W. Smith) Sacc. var. **leteius** n. var. (Figs. 1D$_2$ and 43; Pl. iv, figs. 1, 2, 10; Pl. v, fig. 9)

Differs from *F. culmorum* mainly by somewhat comma-like conidia from aërial mycelium, by typical presence of medium large (up to from 3 to 5 millimeters in diameter) sporodochia, and by somewhat broader average size of conidia.

Hab. On rotted tubers of *Solanum tuberosum*, Atlanta and Forks, New York.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture fifteen days old; conidia from a sporodochium:

Conidia: 1–septate, 0.5 per cent, 18 x 5.2 (14–22 x 4.7–5.9)μ
2–septate, 5.5 per cent, 23 x 6.9 (20–26 x 6.4–7.9)μ
3–septate, 34 per cent, 26.5 x 7 (21–33 x 5.6–7.6)μ
4–septate, 38 per cent, 33.2 x 7.3 (26–38 x 6.4–7.9)μ
5–septate, 22 per cent, 36.4 x 7.5 (31–44 x 7–8.8)μ
6–septate, very few, 44.5 x 8.9μ (only one measured)

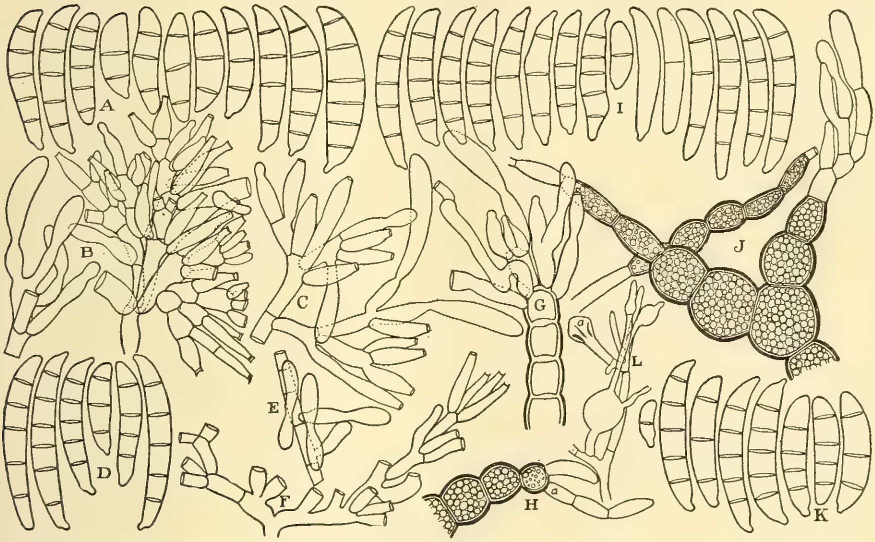

Fig. 43.—*Fusarium culmorum var. leteius.* A, *Conidia,* B, *conidiophores, from aërial sporodochium from 15-days-old culture on slightly acidified hard potato agar;* C, *conidiophore from 11-days-old culture on hard lima-bean agar;* D, *conidia from plectenchymic sporodochium,* E, F, G, *conidiophores, from 72-days-old culture on red raspberry cane plug;* H, *intercalary chlamydospores from 175-days-old culture on corn agar;* I, *conidia from plectenchymic sporodochium from 11-days-old culture on hard lima-bean agar;* J, *intercalary chlamydospores from 175-days-old culture on corn agar (some chlamydospores producing conidiophores);* K, *conidia from aërial sporodochia from 72-days-old culture on red raspberry cane plug;* L, *tip of aërial hypha showing prominent swellings (magnification 250 times)*

On red raspberry cane plug, culture seventy-two days old:

(1) Conidia from a plectenchymic sporodochium borne directly on the plug

Conidia: 3–septate, 14 per cent
4–septate, 20 per cent
5–septate, 66 per cent, 43 x 6.2 (31–53 x 5.8–7)μ

(2) Conidia from a bushlike sporodochium borne on aërial mycelium

Conidia: 1– and 2–septate, very few
3–septate, 40 per cent, 29 x 6.9 (26–35 x 6–7.8)μ
4–septate, 30 per cent, 32 x 6.8 (29–36 x 6–7.5)μ
5–septate, 30 per cent, 37 x 6.8 (31–42 x 6.3–7.5)μ

On hard lima-bean agar, culture eleven days old; conidia from a sporodochium (only mature spores measured):

Conidia: 1–septate, 2 per cent
3–septate, 26 per cent, 32 x 6.1 (24–37 x 5.2–6.7)μ
4–septate, 37 per cent
5–septate, 35 per cent, 39 x 5.9 (30–44 x 5.3–6.7)μ

Average of the above measurements:

Conidia: 1–septate, 0.5 per cent, 18 x 5.2μ
2–septate, 1.5 per cent, 23.5 x 6.9μ
3–septate, 28 per cent, 27.2 x 6.7μ
4–septate, 31 per cent
5–septate, 39 per cent, 38.85 x 6.6μ
6–septate, exceptional, 44.5 x 8.9μ (only one measured)

XI. SECTION MARTIELLA Wr. (Fig. 1, X₁ to C₂). Wollenweber, H. W., Phytopath. **3**:30, Fig. 1, A to C. 1913

Microconidia on aërial mycelium typically present, from oval to oblong, mostly 0–septate; macroconidia mostly 3–septate, of nearly even diameter throughout or but slightly broader either toward apex or toward base, nearly straight in lower half and more or less curved near apex, with somewhat rounded apex or only slightly pointed or constricted, typically from slightly pedicellate to apedicellate; aërial mycelium mostly from white to a light tint of chamois and drab hues; substratum, on neutral agars, never rose-pink nor pomegranate purple in color, sometimes from vinaceous to blue; color of conidia, except in acid media, mostly from pale buff to drab, often distinctly from green to blue.

53. *Fusarium Martii* Ap. et Wr. (Figs. 1, Z₁ and A₂, and 44M)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:78–84, text fig. 5. 1910.

Macroconidia mostly 3–septate, 43.9 x 5.15 (42–46 x 4.9–5.3)μ, and

4–septate, 49.3 x 5.3 (48–50 x 4.9–5.4)μ, often also 5–septate, typically, when mature, of from deep lichen and montpellier green (on corn meal agar) to light olive-drab (on potato agar rich in glucose) and often to dark blue (on potato tuber plug);[56] macroconidia usually produced in abundance in small sporodochia and in pseudopionnotes; chlamydospores terminal and intercalary, single, in clusters, and in short chains, mostly 0–septate, 9.25 x 8.16 (8–11.3 x 7.5–9.3)μ; aërial mycelium typically medium short (from 1 to 4 millimeters), loose, more or less coarsely powdered with conidia, and typically, on potato agar rich in glucose, of from smoke gray to sometimes Chartura drab color; the substratum on the same kind of medium being mostly of from tawny olive to sepia in color.

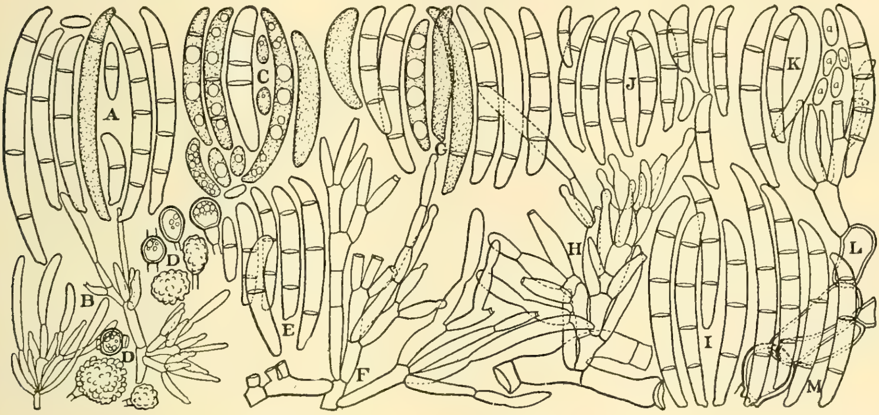

FIG. 44.— A-I, *Fusarium Martii var. viride.* A, *Pseudopionnotal conidia (the stippled one showing dense granulation of the protoplasm masking septation),* B, *conidiophores (magnification 250 times), from 11-days-old culture on slightly acidified hard potato agar;* C, *conidia showing dense granulation of the protoplasm containing from small to large oil globules from 29-days-old culture on rye straw;* D, *chlamydospores, terminal and intercalary,* E, *pseudopionnotal conidia, from 71-days-old culture on potato tuber plug;* F, *conidiophore from 70-days-old culture on potato stem plug;* G, *conidia,* H, *conidiophore, from 47-days-old culture on stem plug;* I, *pseudopionnotal conidia from 64-days-old culture on potato tuber plug*

J-L, *Fusarium Martii var. minus.* J, *conidia from plectenchymic sporodochium from 71-days-old culture on potato tuber plug;* K, *sporodochial conidia from 85-days-old culture on red raspberry cane plug;* Ka, *microconidia from aërial mycelium;* L, *basal part and one branch of compound conidiophore from 71-days-old culture on potato stem plug*

M, *Fusarium Martii, pseudopionnotal conidia from 11-days-old culture on slightly acidified hard potato agar*

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[56] The culture media are mentioned here merely because the colors were oftener observed on these media than on others; in fact, a green color in conidia is often produced also on potato tuber plugs and on some other media, and the same is true of a blue color. The color is due to color of conidia, not to color of substratum alone, as can be observed under the microscope.

Hab. On rotted tubers of *Solanum tuberosum* and on other plants, in the United States and in Europe. The organism was isolated by the writer from specimens received from various States.

Measurements of conidia on different media are as follows:

On potato tuber plug, culture seventy days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, 3 per cent
2–septate, rare
3–septate, 60 per cent, 46 x 5.3 (38–51 x 5.2–5.7)μ
4–septate, 30 per cent, 50 x 5.4 (43–54 x 5.2–5.9)μ
5–septate, 7 per cent, 52 x 5.6 (45–54 x 5.2–5.9)μ

On slightly acidified hard potato agar, culture eleven days old; conidia from thin pseudopionnotes:

Conidia: 0–septate, 2 per cent, 11 x 4μ
1–septate, 2 per cent, 20 x 4.5μ
2–septate, rare
3–septate, 23 per cent, 43.5 x 5.2 (33–51 x 4.6–5.3)μ
4–septate, 68 per cent, 49 x 5.6 (42–65 x 4.6–6.7)μ
5–septate, 5 per cent, 60 x 5.85 (56–63 x 5.6–6.45)μ

On red raspberry cane plug, culture eighty-two days old; conidia from a sporodochium:

Conidia: 1– and 2–septate, rare
3–septate, 57 per cent, 44 x 5.1 (29–50 x 4.3–5.9)μ
4–septate, 43 per cent, 51 x 5.3 (40–54 x 4.7–5.9)μ
5–septate, rare, 54 x 5.4μ (only one measured)

On potato tuber plug, culture sixty-four days old; conidia from thick pseudopionnotes:

Conidia: 0–septate, rare, about 9 x 3.5μ
3–septate, 55 per cent, 43 x 4.9 (28–50 x 4.7–5.1)μ
4–septate, 45 per cent, 48 x 4.9 (43–55 x 4.7–5.7)μ
5–septate, very rare

On hard lima-bean agar, culture sixty-eight days old; conidia from a small sporodochium:

Conidia: 0– to 2–septate, very rare
3–septate, 48 per cent, 42 x 5.2 (30–51 x 4.8–5.9)μ
4–septate, 50 per cent, 48 x 5.3 (41–53 x 4.8–5.9)μ
5–septate, 2 per cent, 51 x 5.5 (43–56 x 5–5.9)μ

On same medium and of same age as above, conidia from a medium large (about 2 millimeters in diameter), short, column-like sporodochium:

Conidia: 0– to 2–septate, very rare
3–septate, 49 per cent, 45 x 5.2 (36–49 x 4.5–5.6)μ
4–septate, 51 per cent, 50 x 5.2 (38–56 x 4.8–5.6)μ
5–septate, very rare

Average of the above measurements:

Conidia: 0–septate, rare, 11 x 4μ
1–septate, 1 per cent, 20 x 4.5μ
2–septate, very rare
3–septate, 53 per cent, 43.9 x 5.15μ
4–septate, 43 per cent, 49.3 x 5.3μ
5–septate, 3 per cent, 54.3 x 5.57μ

The average sizes given by Appel and Wollenweber for the same fungus are:

Conidia: 3–septate, about 44 per cent, 49 x 5.25μ
4–septate, about 51.5 per cent, 55 x 5.5μ
5–septate, about 4.5 per cent, 56.5 x 5.5μ

54. *Fusarium Martii* Ap. et Wr. var. **viride** n. var. (Fig. 44, A to I; Pl. VI, fig. 5)

Differs from *F. Martii* by having macroconidia somewhat narrower, and usually by a paler color of conidia and substratum; dark blue color of conidial masses not observed. Typical color of conidia in mass, on potato agar rich in glucose, pale smoke-gray, and of substratum pale drab-gray.

Hab. In discolored fibrovascular bundles of *Solanum tuberosum*, in stems and tubers, at Atlanta and Castile, New York.

Measurements of conidia on various media are as follows:

On potato tuber plug, culture seventy-one days old; conidia from pseudopionnotes:

Conidia: 0–septate, 4 per cent
1–septate, 22 per cent
2–septate, 10 per cent
3–septate, 60 per cent, 39 x 5 (33–43 x 4.6–5.2)μ
4–septate, 4 per cent

On slightly acidified hard potato agar, culture eight days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare, 9 x 4μ
1–septate, rare, 17 x 5μ
2–septate, rare
3–septate, 41 per cent, about 46 x 5 (42–51 x 4.4–5.25)μ
4–septate, 58 per cent, 54.4 x 5.25 (45–68 x 4.8–5.6)μ
5–septate, 1 per cent, 58.5 x 5.7 (50–62 x 5–6)μ

On red raspberry cane plug, culture eighty-six days old; conidia from a sporodochium:

Conidia: 3–septate, 50 per cent, 45 x 5.2 (36–53 x 5–5.4)μ
4–septate, 50 per cent, 54 x 5.4 (42–61 x 5.2–5.9)μ

On hard lima-bean agar, culture twenty-two days old; conidia from pseudopionnotes:

Conidia: 3–septate, 90 per cent, 46.1 x 5 (40–49 x 4.7–5.3)μ
4–septate, 9 per cent
5–septate, 1 per cent

On potato tuber plug, culture sixty-four days old; conidia from thick pseudopionnotes:

Conidia: 3–septate, 55 per cent, 45 x 4.9 (40–49 x 4.8–5.3)μ
4–septate, 45 per cent, 49 x 5.3 (45–53 x 5–5.6)μ

On hard lima-bean agar, culture seventy-two days old; conidia from a columnar sporodochial mass:

Conidia: 3–septate, 73 per cent, 45 x 5.1 (40–49 x 4.7–5.3)μ
4–septate, 27 per cent, 50 x 5.2 (47–51 x 5–5.6)μ

Average of the above measurements:

Conidia: 0–septate, 0.5 per cent, 9 x 4μ
1–septate, 3 per cent, 17 x 5μ
2–septate, 1 per cent
3–septate, 63 per cent, 44.4 x 5.03μ
4–septate, 32 per cent, 51.9 x 5.27μ
5–septate, 0.5 per cent, 58.5 x 5.7μ

55. *Fusarium Martii* Ap. et Wr. var. **minus** n. var. (Fig. 44, J to L; Pl. 1, figs. 3 and 4; Pl. VI, fig. 6)

Differs from *F. Martii* and *F. Martii* var. *viride* by having smaller, 3–septate, conidia, 36.7 x 4.8 (30–44 x 4.55–5.1)μ, usually prominent development of plectenchymic, wartlike stromata, and fewer and larger sporodochia (Pl. VI, fig. 6).

Color of substratum, on potato agar rich in glucose, from light gray to drab and dark olive-buff, with a fuscous-colored spot at the point of inoculation (Pl. I, figs. 3 and 4).

Hab. On rotted tubers of *Solanum tuberosum*, evidently following *Phytophthora infestans*, Dutchess County, New York.

Measurements of conidia on different media are as follows:

On potato tuber plug, culture seventy-one days old:

(1) Conidia from a sporodochium

Conidia: 0–septate, 8 per cent
1–septate, 13 per cent
2–septate, 29 per cent
3–septate, 50 per cent, 30 x 4.6 (26–33 x 3.6–4.8)μ

(2) Conidia from pseudopionnotes

Conidia: 1–septate, 9 per cent
2–septate, 37 per cent
3–septate, 54 per cent, 30 x 4.55 (27–34 x 3.9–4.8)μ

(3) Conidia from oldest part of pseudopionnotes

Conidia: 3–septate, 100 per cent, 37 x 4.7 (27–43 x 4.2–5)μ

On slightly acidified hard potato agar, culture eleven days old; conidia from pseudopionnotes:

Conidia: 3–septate, 67 per cent, 43.75 x 4.6 (24–52 x 3.5–5.3)μ
4–septate, 28 per cent, 48.65 x 5 (43–53 x 4.3–5.3)μ
5–septate, 5 per cent, 52.85 x 5 (50–57 x 4.3–5.3)μ

On red raspberry cane plug, culture eighty-five days old; conidia from a sporodochium:

Conidia: 0–septate, rare
1–septate, rare
2–septate, rare
3–septate, 80 per cent, 38 x 5.1 (28–48 x 4.1–5.3)μ
4–septate, 20 per cent, 47 x 5.2 (42–52 x 4.9–5.5)μ
5–septate, rare, 47 x 5.2μ (only one measured)

On hard lima-bean agar with 2 per cent glucose, culture twenty-two days old:

Conidia: 3–septate, 88 per cent, 41.4 x 4.7 (36–47 x 4.1–5.3)μ
4–septate, 10 per cent
5–septate, 2 per cent

On potato tuber plug, culture sixty-four days old; conidia from pseudopionnotes:

Conidia: 0–septate, very rare
3–septate, 86 per cent, 40 x 4.8 (33–49 x 4.1–5.2)μ
4–septate, 14 per cent, 48 x 4.9 (38–56 x 4.7–5.7)μ

On hard lima-bean agar with 2 per cent glucose, culture sixty-eight days old; conidia from a columnar mass:

Conidia: 0– to 2–septate, very rare
3–septate, 93 per cent, 40 x 5.1 (30–45 x 4.7–5.4)μ
4–septate, 7 per cent, 44 x 5.1 (36–48 x 4.7–5.4)μ
5–septate, very rare, 47 x 5.3μ (only one measured)

On same medium, culture also same age; conidia from pseudopionnotes:

Conidia: 0– to 2–septate, very rare
3–septate, 98 per cent, 41 x 5 (33–42 x 4.7–5.3)μ
4–septate, 2 per cent, 44 x 5.1 (40–50 x 4.8–5.3)μ

Average of the above measurements:

Conidia: 0–septate, 11 per cent
1–septate, 2 per cent
2–septate, 6.6 per cent
3–septate, 71.6 per cent, 36.7 x 4.8μ
4–septate, 8.1 per cent, 46.2 x 5.06μ
5–septate, 0.7 per cent, 50 x 5.17μ

56. *Fusarium Solani* (Mart. p. par.) Ap. et Wr. (Fig. 1, X_1 and Y_1; Fig. 45, A to G; Pl. I, fig. 1; Pl. VI, fig. 7)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:64–78; Pl. I, figs. 1 to 30; Pl. III, fig. 1; text fig. 4. 1910.

Microconidia always present, at least on aërial mycelium, same size and shape as those of *F. Martii*. Macroconidia typically somewhat broader

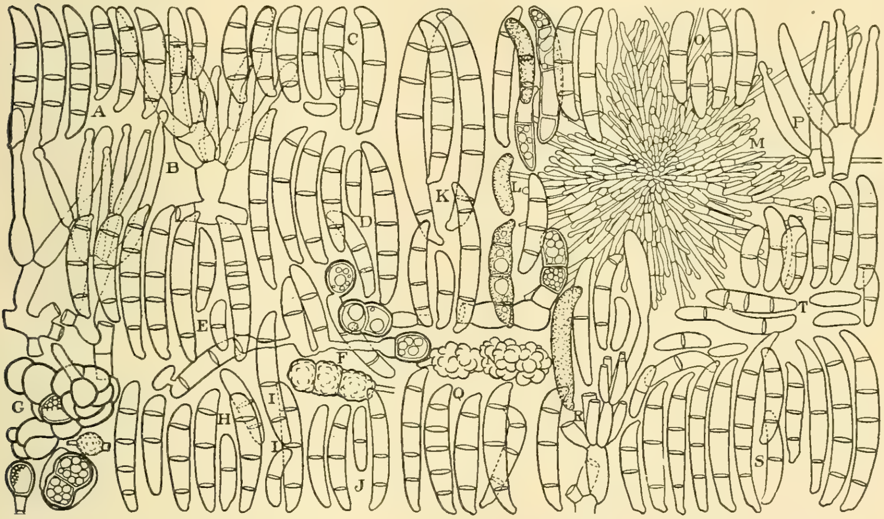

FIG. 45.— A-G, *Fusarium Solani.* A, *Conidia from plectenchymic sporodochia from 66-days-old culture on potato tuber plugs;* B, *conidiophores,* C, *conidia, from aërial mycelium from 12-days-old culture on hard lima-bean agar;* D, *pseudopionnotal conidia from 11-days-old culture on slightly acidified hard potato agar;* E, *conidia from sporodochium from 87-days-old culture on red raspberry cane plug;* F, *terminal, 1- to 3-septate, chlamydospores produced by hyphæ and conidia, the latter anastomosed, from 173-days-old culture on corn agar;* G, *terminal and intercalary, clustered, chlamydospores from 70-days-old culture on potato tuber plug*

H-J, *Fusarium Solani var. cyanum.* H, *Sporodochial conidia from 87-days-old culture on red raspberry cane plug;* I, *pseudopionnotal conidia from 15-days-old culture on slightly acidified hard potato agar;* J, *aërial conidia from 87-days-old culture on red raspberry cane plug*

K-T, *Fusarium Solani var. suffuscum.* K, *Pseudopionnotal conidia from 7-days-old colony in petri dish on hard potato agar (the middle spore above is typical for the culture);* L, *sporodochial conidia from 47-days-old culture on rye straw (many conidia have coarsely granulated protoplasm and oil globules);* M, *compound conidiophore in form of dense tuft (looking from above, magnification 250 times), from 47-days-old culture on potato stem plug;* N, *chlamydospores,* O, *sporodochial conidia, from 70-days-old culture on potato tuber plug;* P, *typical ends of conidiophores from 47-days-old culture on potato stem plug;* Q, *sporodochial conidia from 87-days-old culture on red raspberry cane plug;* R, *sporodochial conidia and conidiophore from 13-days-old culture on potato tuber plug;* S, *sporodochial conidia from 16-days-old culture on slightly acidified hard potato agar;* T, *sporodochial conidia from 47-days-old culture on potato stem plug* in upper half of their length, with from rounded to slightly constricted apex, not at all or slightly pedicellate, typically 3–septate, 29.75 x 5.5 (27–34.7 x 5.4–5.8)μ, sometimes 4–septate, rarely 5–septate; aërial mycelium from poorly to well developed, from white to olive-buff; substratum, on potato agar rich in glucose, olive–buff with a green–blue tinge (Pl. I, fig, 1, and Pl. VI, fig. 7).

Hab. On *Solanum tuberosum* and other substrata, in Europe and America; often in association with other Fusaria.

The organism was often isolated by the writer from rotted potato tubers received from various States.

Measurements of conidia on various media are as follows:

On slightly acidified hard potato agar, culture eleven days old; conidia from pseudopionnotes:

Conidia: 1–septate, 0.5 per cent, 14 x 4 (13–17 x 3.5–5.8)μ
2–septate, 1 per cent, 22 x 4.5 (17–28 x 4.2–4.8)μ
3–septate, 93 per cent, 34.7 x 5.4 (22–42 x 4.3–6.1)μ
4–septate, 5 per cent, 38 x 5.8 (30–43 x 5–6.4)μ
5–septate, 0.5 per cent, 44.3 x 5.8 (38–47 x 5–6.4)μ

On potato tuber plug, culture sixty-five days old; conidia from a sporodochium:

Conidia: 0–septate, rare, 8.7 x 4μ (only a few measured)
1–septate, 2 per cent, 20 x 4.6μ
2–septate, 4 per cent, 23 x 4.7 (18–25 x 4.4–5.4)μ
3–septate, 93 per cent, 28 x 5.8 (22–35 x 4.8–6.1)μ
4–septate, 1 per cent, 33–5.9 (28–38 x 5.4–6.1)μ

On hard lima-bean agar, culture twelve days old:

(1) Conidia from aërial mycelium

Conidia: 0–septate, 20 per cent
1–septate, 40 per cent
2–septate, 19 per cent
3–septate, 21 per cent, 27 x 5.4 (24–37 x 4.7–6)μ

(2) Conidia from a sporodochium

Conidia: 0–septate, 1 per cent
1–septate, 1 per cent
2–septate, 1 per cent
3–septate (97 to 100 per cent), 29.3 x 5.4 (24–35 x 4.8–5.9)μ

Average of the above measurements:
Conidia: 0–septate, 5 per cent, 8.7 x 4μ
1–septate, 11 per cent, 17 x 4.3μ
2–septate, 6 per cent, 22.5 x 4.6μ
3–septate, 76.5 per cent, 29.75 x 5.5μ
4–septate, 1.5 per cent, 35.5 x 5.85μ
5–septate, rare, 44.3 x 5.8μ

57. *Fusarium Solani* (Mart. p. par.) Ap. et Wr. var. **cyanum** n. var. (Fig. 45, H to J)

Differs from *F. Solani* mainly by having macroconidia typically more rounded at apex and base, by fewer 4–septate and absence of 5–septate conidia, and by frequent occurrence of bluish plectenchyma (on hard bean agar and potato tuber plugs); sometimes distinct blue color in conidia also observable; size for the same septation type of conidia almost the same as that of *F. Solani*.

Hab. Same as for *F. Solani*, but much less common.

On potato tuber plug, culture sixty-eight days old; conidia from a sporodochium:
Conidia: 0–septate, 28 per cent
1–septate, 22 per cent
2–septate, 35 per cent
3–septate, 15 per cent, 28 x 5.2 (24–31 x 4.8–5.7)μ

On slightly acidified hard potato agar, culture fifteen days old; conidia from pseudopionnotes:
Conidia: 0–septate, 14 per cent, 12 x 3.7 (8.7–14 x 2.5–5)μ
1–septate, 22 per cent, 24.5 x 4.5 (15–30 x 3.5–5.3)μ
2–septate, 19 per cent, 29 x 5 (24–32 x 4.8–5.3)μ
3–septate, 45 per cent, 33 x 5.7 (31–35 x 5–6)μ

On red raspberry cane plug, culture eighty-seven days old; conidia from a sporodochium:
Conidia: 0–septate, few
1–septate, 3 per cent
2–septate, 5 per cent, 25 x 5.5 (19–27 x 4.7–5.8)μ
3–septate, 90 per cent, 30 x 5.7 (28–35 x 5.2–5.9)μ
4–septate, 2 per cent, 35 x 5.5 (33–37 x 5.2–5.7)μ

On potato tuber plug, culture sixty-five days old; conidia from a sporodochium:

Conidia: 0–septate, few
1–septate, 12 per cent, about 22 x 5μ (only one measured)
2–septate, 16 per cent, about 27 x 5.3μ (only one measured)
3–septate, 72 per cent, 29 x 5.6 (24–35 x 4.8–5.9)μ

On corn agar, culture one hundred and seventy-five days old; conidia only few, chlamydospores numerous, terminal and intercalary:

Chlamydospores: 0–septate, in mycelium, 11.3 x 9.3 (8.5–16 x 7.5–11)μ
1–septate, in mycelium, 16.3 x 9.9 (14–20 x 8–14)μ

On hard lima-bean agar, culture twelve days old; conidia from pseudopionnotes:

Conidia: 0–septate, 4 per cent
1–septate, 15 per cent
2–septate, 16 per cent
(percentage may vary considerably from somewhat greater than figures given here to nearly zero)
3–septate, 65 per cent, 33 x 5.2 (27–37 x 4.7–5.6)μ

Average of the above measurements:

Conidia: 0–septate, 9 per cent, 12 x 3.7μ
1–septate, 15 per cent, 23 x 4.75μ
2–septate, 18 per cent, 27 x 5.3μ
3–septate, 57.5 per cent, 30.5 x 5.5μ
4–septate, 0.5 per cent, 35 x 5.5μ

58. *Fusarium Solani* (Mart. p. par.) Ap. et Wr. var. **suffuscum** n. var. (Fig. 45, K to T)

Differs from *F. Solani* and *F. Solani* var. *cyanum* mainly by typically well-developed, uniform, fine, aërial mycelium, with mass of chlamydospores at maturity which gives it a pale brownish tint; by sparse conidial production on aërial mycelium; and by the fact that sporodochia are usually few and distant from one another.

Hab. On rotted tubers of *Solanum tuberosum*, Wisconsin.

The average size and occurrence of septation type of conidia on various media are as follows:

Conidia: 0–septate, 5.5 per cent, 10.7 x 3.15μ
1–septate, 13.5 per cent, 20 x 4.5μ
2–septate, 17 per cent, 25 x 4.5μ
3–septate, 60.5 per cent, 30 x 5.41μ
4–septate, 3.5 per cent, 34.5 x 5.5μ
5–septate, rare, 42.25 x 5.85μ

59. **Fusarium striatum** n. sp. (Figs. 1c_2 and 46; Pl. I, fig. 2)

Microconidia, at least on aërial mycelium, always present. Macroconidia of shape and septation intermediate between *F. Martii* and *F. Solani*, mostly 3–septate, 34.7 x 4.6 (31–36 x 4.4–5)μ, from colorless to yellowish glaucous and pale turquoise green, in numerous minute sporodochia; sporodochia often converging into a pseudopionnotes; aërial mycelium short (rarely up to 3 millimeters high), typically (on various agars) fine, uniformly from loose to very loose, downy in appearance, from white to grayish white; substratum, on agars rich in glucose, from pale glaucous green to tawny olive and sepia.

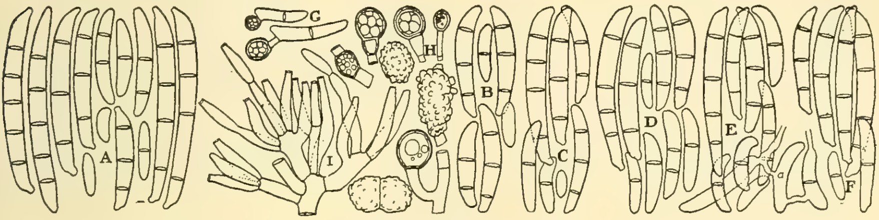

FIG. 46.—*Fusarium striatum.* A, *Pseudopionnotal conidia from 17-days-old culture on slightly acidified hard potato agar;* B, *pseudopionnotal conidia from 58-days-old culture on oats;* C, *conidia from small sporodochium from 32-days-old culture on hard lima-bean agar;* D, *sporodochial conidia from 86-days-old culture on red raspberry cane plug;* E, *pseudopionnotal conidia from 13-days-old potato tuber plug (some of them anastomosing);* F, *pseudopionnotal conidia from 12-days-old culture on hard lima-bean agar;* G, *terminal chlamydospores produced by conidia from 64-days-old culture on hard lima-bean agar with 2 per cent glucose;* H, *chlamydospores from 32-days-old culture on hard lima-bean agar;* I, *compound conidiophore from 34-days-old culture on hard lima-bean agar*

Hab. On tubers of *Solanum tuberosum*, Colorado.

Latin description.—Microconidiis — saltem in aerio mycelio — semper praesentibus; macroconidiis forma et septatione inter F. Martii et F.

Solani mediis, plerumque 3–septatis, 34.7 x 4.6 (31–36 x 4.4–5)μ, ex hyalino luteolo-glaucis vel pallide "turquoise green" (R), multis minutis sporodochiis; sporodochiis saepe in pseudopionnotem vergentibus; aerio mycelio brevi (usque ad 3 mm. alt.) typice (in agaribus variis) subtili, aequabiliter laxo vel laxissimo, pubescenti viso, ex albo caesio-albo; substrato — in agaribus perglucosis — ex pallide glauco-viridi "tawny-olive" (R) et "sepia" (R).

Hab. In tuberibus Solani tuberosi, Colorado, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture seventeen days old; conidia from pseudopionnotes:

Conidia: 0–septate, 6 per cent, 10.6 x 3.15 (8.5–12.5 x 2.5–3.5)μ
1–septate, 10 per cent, 19.5 x 3.9 (14–21 x 3–4.4)μ
2–septate, 4 per cent, 24.15 x 4.2 (21–27 x 3.5–4.7)μ
3–septate, 64 per cent, 36.4 x 4.4 (26–50 x 4–5)μ
4–septate, 16 per cent, 47 x 4.5 (40–56 x 4.3–5.3)μ
5–septate, rare, 50 x 4.8 (47–56 x 4.3–5.3)μ

On red raspberry cane plug, culture eighty-six days old; conidia from a sporodochium:

Conidia: 0–septate, 3 per cent
1–septate, 7 per cent, 19 x 3.5 (12–23 x 3–4.1)μ
2–septate, 2 per cent
3–septate, 88 per cent, 41.6 x 5 (24–44 x 4–5.7)μ
4–septate, very rare, 43 x 5 (only one measured)

On hard lima-bean agar with 2 per cent glucose, culture sixty-four days old; conidia from pseudopionnotes:

Conidia: 0–septate, 10 per cent
1–septate, 22 per cent, 22 x 4.1 (16-30 x 3.5–4.7)μ
2–septate, 10 per cent
3–septate, 58 per cent, 31 x 4.6 (22–37 x 4.2–5)μ

On rye grain, culture sixty-four days old; conidia from a sporodochium:

Conidia: 0–septate, 39 per cent
1–septate, 28 per cent, 20 x 3.9 (14–23 x 3.5–4.4)μ
2–septate, 5 per cent
3–septate, 28 per cent, 31 x 4.6 (24–36 x 4.3–5)μ

On hard lima-bean agar, culture twelve days old:

(1) Conidia from a sporodochium
- 0–septate, 13 per cent
- 1–septate, 30 per cent
- 2–septate, 7 per cent
- 3–septate, 50 per cent, 33 x 4.4 (24–44 x 3.6–4.7)μ
- 4–septate, very few

(2) Chlamydospores, terminal and intercalary, mostly 0–septate, 9 x 7.5 (7–11 x 6–9)μ

On same medium as above, culture thirty-two days old; conidia from a sporodochium:

Conidia: 0–septate, 1 per cent, 9 x 3.6μ (only a few measured)
1–septate, 20 per cent, 20 x 3.9 (14–25 x 3.5–4.1)μ
2–septate, 5 per cent, 25 x 4.2 (23–30 x 4–4.4)μ
3–septate, 73 per cent, 35 x 4.6 (28–37 x 4–4.7)μ
4–septate, 1 per cent, 41 x 4.7μ (only a few measured)

Average of the above measurements:

Conidia: 0–septate, 12 per cent, 10 x 3.4μ
1–septate, 19.5 per cent, 20 x 3.9μ
2–septate, 5.5 per cent, 24.5 x 4.2μ
3–septate, 60 per cent, 34.7 x 4.6μ
4–septate, 3 per cent, 43.7 x 4.7μ
5–septate, very rare, 50 x 4.8μ

60. *Fusarium radicicola*[57] Wr. (Fig. 47; Pl. VI, fig. 8)

Wollenweber, H. W., Journ. Agr. Research **2**:257-258, Pl. XVI, fig. K, 1914.

Microconidia nearly straight near base, slightly curved in upper third of their length, with from somewhat rounded to distinctly constricted apex, slightly pedicellate, mostly 3–septate, 35.2 x 4.7 (31–40 x 4.6–5)μ; 0– and 1–septate, microconidia very common, 0–septate measuring 8 x 3μ; chlamydospores common, terminal and intercalary, mostly 0– and 1–septate, 0–septate averaging 9–10 x 8.7–8.8μ; pseudopionnotes typically absent; plectenchymic sporodochia often present; aërial mycelium well developed; color of conidia from white to olive, of substratum from pale yellowish to olive (on agar rich in glucose and on potato tuber plug).

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[57] In an article which appeared after this work was ready for press, Dr. Wollenweber (1914) describes a new species of Fusarium (*F. radicicola*) with which this organism appears identical. No cultural comparisons have been possible, but the type of tuber rot and type of conidia are alike, and in certain instances material for study has come from the same region. Possibly any differences may be accounted for in the difference in strains studied.

Hab. On rotted tubers of *Solanum tuberosum,* in Oregon, Idaho, and California.

F. radicicola differs from *F. Martii* and *F. Martii* var. *viride* by shorter macroconidia; from *F. Martii* var. *minus* by absence of prominent plectenchymic sporodochia; and from *F. striatum* by well-developed aërial mycelium and by typical absence of pseudopionnotes.

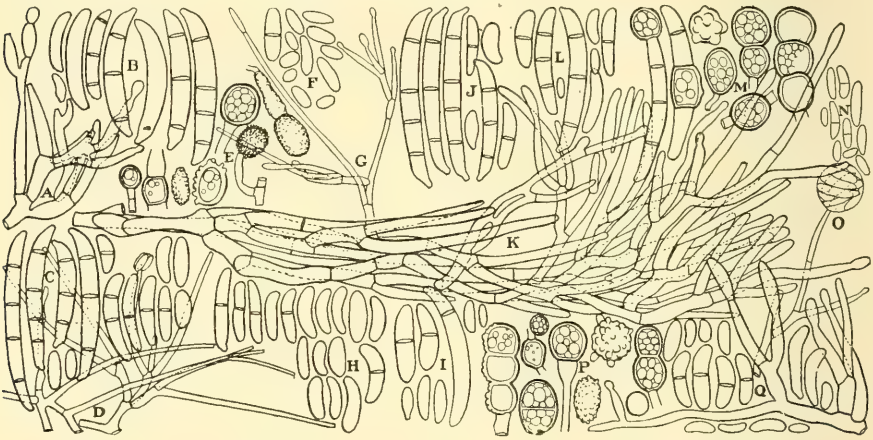

FIG. 47.—*Fusarium radicicola.* A, *Conidiophore,* B, *sporodochial conidia, from 67-days-old culture on potato stem plug;* C, *aërial and pseudopionnotal conidia from 12-days-old culture on hard lima-bean agar;* D, *conidiophore (magnification 250 times) from 48-days-old culture on rye straw;* E, *chlamydospores,* F, *aërial conidia,* G, *conidiophore (magnified 250 times), from 73-days-old culture on red raspberry cane plug;* H, *conidia from the surface of original stem-end rotted potato tuber;* I, *conidia from 48-days-old culture on rye straw;* J, *conidia from pseudopionnotes in 7-days-old colony in petri dish on hard potato agar;* K, *conidiophore from the surface of original stem-end rotted potato tuber;* L, *aërial conidia close to substratum from 64-days-old culture on hard lima-bean agar;* M, *chlamydospores produced in mycelium and conidia from 173-days-old culture on corn agar;* N, *conidia,* O, *spore ball, both magnified 250 times, from 3-days-old hanging drop culture in potato decoction in van Tieghem cell;* P, *chlamydospores from 82-days-old culture on potato tuber plug;* Q, *conidia and conidiophores from 26-days-old culture on oats*

Production of macroconidia in sporodochia was observed to be abundant only under certain conditions (not well determined as yet), but usually microconidia alone are the dominant type. The Oregon specimens, from which cultures of the fungus were first obtained, showed a dark, depressed area of dry rot at the stem end of the tubers, with cream-white, dense tufts of conidiophores, up to 1 centimeter high and powdered with conidia. (For microscopic characters see figure 47.) Pure cultures of this organism were obtained both from the aërial conidia and from plantings of the rotted tissues of the tubers.

The organisms in general differ much from *F. striatum*, but in certain cultures (in sporodochia-producing stage on whole steamed potato tubers) resemble it very closely.

Measurements of conidia on different media are as follows:

On potato tuber plug, culture eighty-two days old:
(1) Conidia from aërial mycelium
Conidia: 0–septate, 95 per cent, 8 x 3 (6–15 x 2.1–4)μ
1–septate, 5 per cent, 15 x 4 (13–21 x 3.4–4.4)μ
3–septate, rare
(2) Chlamydospores, intercalary and terminal, unicellular and in chains
0–septate, 9.8 x 8.7 (5.2–16 x 5.2–12)μ

On hard lima-bean agar with 2 per cent glucose, culture sixty-four days old:
(1) Conidia from aërial mycelium close to substratum
Conidia: 0–septate, 80 per cent
1–septate, 17 per cent } (size same as above)
2–septate, 2 per cent
3–septate, 1 per cent, 33 x 4.8 (22–41 x 4.3–5.2)μ
4–septate, rare, 45 x 4.8 (only a few measured)
(2) Chlamydospores
0–septate, 9.5 x 8.8 (6.1–12 x 5.2–11)μ
1–septate, 18 x 10 (16–22 x 5–12)μ

On hard lima-bean agar, culture twelve days old:
(1) Conidia from aërial mycelium
Conidia: 0–septate, 60 per cent
1–septate, 30 per cent
2–septate, 1 per cent
3–septate, 6 per cent, 37 x 4.5 (28–48 x 4–5.3)μ
4–septate, 3 per cent, 43.6 x 5 (40–49 x 4.3–5.2)μ
5–septate, rare, size about that of 4–septate
(2) Chlamydospores, mostly 0–septate, 9 x 8.7μ

On whole steamed potato tuber, culture sixty days old; conidia from a sporodochium:

Conidia: 0–septate, 11 per cent
1–septate, 21 per cent
2–septate, 8 per cent
3–septate, 60 per cent, 31 x 4.8 (29–38 x 4.2–5.5)μ
4–septate, very rare

On oat grain, culture fifty-seven days old; conidia from a sporodochium, septation as above:

Conidia: 3–septate, 35.2 x 4.7 (25–42.5 x 4.2–5)μ
4–septate, 43 x 5μ (only one measured)

On potato stem plug, culture seventy days old; conidia from a sporodochium, septation approximately that given above:

Conidia: 3–septate, 35.2 x 5 (24.5–38 x 3.7–5.5)μ
4–septate, 41.6 x 5.8μ (only one measured)

On hard potato agar, culture ten days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 30 per cent
1–septate, 10 per cent
2–septate, 5 per cent
3–septate, 50 per cent, 40 x 4.6 (32–46 x 4.3–4.9)μ
4–septate, 5 per cent, 47 x 4.8 (45–49 x 4.5–5)μ

Average of the above measurements:

Conidia: 0–septate, 56 per cent (from 11 to 95 per cent), 8 x 3μ
1–septate, 16 per cent (from 5 to 30 per cent), 15 x 4μ
2–septate, 3 per cent (from 0 to 8 per cent)
3–septate, 23 per cent (from 0 to 60 per cent), 35.2 x 4.73μ
4–septate, 2 per cent (from 0 to 5 per cent), 43.9 x 5.1μ
5–septate, very rare, size about that of 4–septate

61. *Fusarium coeruleum* (Lib.) Sacc. (Figs. 1B$_2$ and 48; Pl. I, figs. 5 and 6; Pl. VI, fig. 4)

Syn. *Fusarium violaceum* Fuckel. 1869.
Selenosporium coeruleum Libert, in herbarium.

Cf. Saccardo, Syll. Fung. **4**:705. 1886. Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:84–91, Pl. III fig. 6, text fig. 6. 1910. Wollenweber, H. W., Phytopath. 3:31, 44, 45, Fig. 1c. 1913.

Microconidia of larger size than those of other species of section Martiella, 0–septate, about 16 x 4.7μ. Macroconidia, for the largest part, of an even diameter or somewhat broader toward the base, only slightly curved near, and more or less rounded at, the apex, never apically constricted, mostly apedicellate or with ventrally depressed basal cell, mostly 3–septate, 33.3 x 5 (30–36 x 4.5–5.4)μ; aërial mycelium usually medium well developed, feltlike in age, of from white, bluish white, and olive-buff to dusky slate violet, on potato agar rich in glucose, and to slate purple on corn meal agar; substratum, on potato agar rich in glucose, from deep hyssop violet to indian lake, ocher-red, and, in older cultures, violet-carmine; color of conidia from orchraceous orange, on strong acid agars, to pale buff and mouse gray, or often blue, on neutral media.

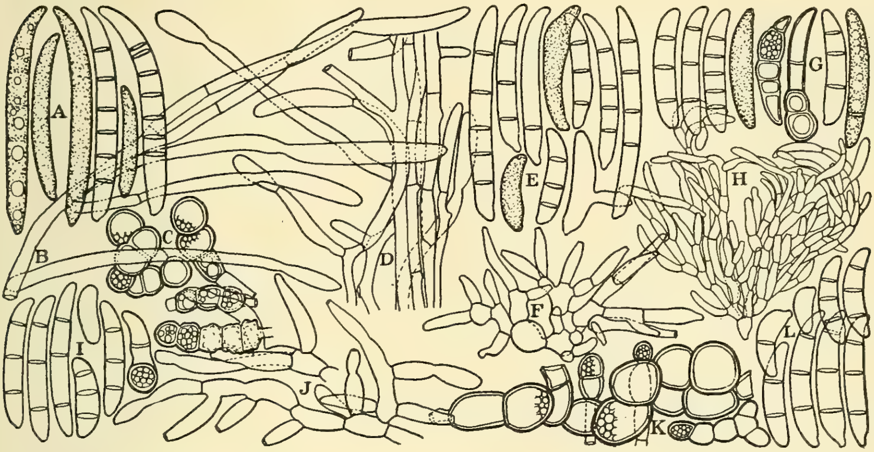

Fig. 48.—*Fusarium coeruleum.* A, *Pseudopionnotal conidia,* B, *conidiophore, from 8-days-old colony in petri dish on hard potato agar;* C, *chlamydospores,* D, *coremium-like form of mycelial growth with conidiophores,* E, *pseudopionnotal conidia, from 35-days-old culture on potato tuber plug;* F, *conidiophore from 62-days-old culture on rye straw;* G, *conidia (some with chlamydospores),* H, *conidiophore (magnified 250 times), from 54-days-old culture on rye straw;* I, *conidia from small sporodochium,* J, *conidiophore, from 79-days-old culture on red raspberry cane plug;* K, *chlamydospores,* L, *pseudopionnotal conidia, from 22-days-old culture on hard potato agar.* A, B, F, G, *and* H *are from strains isolated from rotted potato tubers received from American sources, the remainder are from the culture received from Dr. Wollenweber*

Hab. On rotted tubers of *Solanum tuberosum,* common in Europe and

America. Cause of most of Fusarial rot on potatoes in storage, often occurring in association with other Fusaria.

The organism was repeatedly isolated by the writer from diseased tubers, and every strain isolated proved capable of producing more or less dark-colored dry rot on tubers. It may be mentioned here that a white rot of tubers can be produced by certain species of Fusarium of the Elegans section, such as *F. lutulatum.* A rot distinctly striate in appearance was often produced by inoculation with *F. striatum*; a brownish rot of tubers is caused by *F. trichothecioides*; and a more or less pinkish rot with large cavities, more or less covered with red masses of macroconidia, is produced by *F. subulatum* and *F. subulatum* var. *brevius.*

Of all these, *F. coeruleum* is the commonest and most vigorous wound parasite of potato tubers; the next, perhaps, is *F. subulatum* and its variety.

Measurements of conidia of a few strains of the species on different media are as follows:

On slightly acidified hard potato agar, culture twenty-two days old; conidia from pseudopionnotes, strain 66:

Conidia: 0–septate, 5 per cent, 16 x 4.7 (6–28 x 4.3–5.5)μ
1–septate, 9 per cent, 21 x 4.7 (17–27 x 4.4–5.3)μ
2–septate, 10 per cent, 25 x 5.3 (18–32 x 4.6–5.9)μ
3–septate, 75 per cent, 30 x 5.4 (20–40 x 4.7–6.1)μ
4–septate, 1 per cent, 36 x 5.5 (33–40 x 5.2–6.1)μ

On red raspberry cane plug, culture seventy-nine days old; conidia from a sporodochium, strain 66; 3–septate, up to 100 per cent, 33 x 5.4 (22–40 x 4.8–6)μ; average septation being

Conidia: 0–septate, 3 per cent
1–septate, 11 per cent
2–septate, 8 per cent
3–septate, 78 per cent
4–septate, rare

On corn meal agar, culture one hundred and seventy-five days old; strain 53; very few conidia observed; chlamydospores numerous:

Chlamydospores: 0–septate, 8 x 7.5 (5.2–10.5 x 5.2–9)μ
1–septate, 11.7 x 7.7 (9.2–14 x 7–8.5)μ
Also in chains in mycelium, 9 (7.8–11)μ in diameter

On hard potato agar, culture one hundred and fifty days old; conidia from a sporodochium, strain 96:

Conidia: 0–septate, 10 per cent
1–septate, 12 per cent
2–septate, 7 per cent
3–septate, 71 per cent, 36 x 5.1 (33–40 x 4.4–5.5)μ
Chlamydospores: 0–septate, in conidia, average size 8.8 x 8μ

On potato tuber plug, culture eighty-five days old; conidia from a pseudopionnotes, strain 66:

Conidia: 0–septate, 5 per cent
1–septate, 8 per cent
2–septate, 4 per cent
3–septate, 68 per cent, 34.2 x 4.5μ
4–septate, 9 per cent, 42 x 4.6μ (only one measured)
5–septate, 6 per cent, 55 x 5.5μ (only two measured)

On rye straw, culture fifty-four days old; conidia from a sporodochium, strain 96:

Conidia: 0–septate, 2 per cent
1– to 2–septate, 5 per cent
3–septate, 89 per cent, 33.5 x 5.1 (30–37 x 4–6)μ
4–septate, 4 per cent, 33 x 6.1μ (only one measured)

On rye straw, culture fifty days old; conidia from a sporodochium, strain 66:

Conidia: 0– to 2–septate, 5 per cent
3–septate, 88 per cent, 30.5 x 4.9 (28–33 x 4.5–5.3)μ (only three measured)
4–septate, 7 per cent, 33.2 x 5.1μ (only one measured)

On rye straw, culture forty-three days old; conidia from a sporodochium, strain 53:

Conidia: 0– to 2–septate, 23 per cent
3–septate, 73 per cent, 35 x 5 (25–46.2 x 4.7–5.3)μ
4–septate, 4 per cent, 50 x 5μ (only one measured)

On potato hard agar, culture eight days old; strain 190:

Conidia: 0– to 2–septate, 2 per cent
3–septate, 80 per cent
4– and 5–septate, 10 per cent
6– and 7–septate, 8 per cent, 58 x 5.6 (57–60 x 5.2–6)μ

The greatest septation observed was in a culture fifteen days old on oat grains. This was a 9–septate conidium measuring 39 x 5.2μ.

Average of the above measurements:

Conidia: 0–septate, about 4 per cent, 16 x 4.7μ
1–septate, about 6 per cent, 21 x 4.7μ
2–septate, about 5 per cent, 25 x 5.3μ
3–septate, about 78 per cent, 33.3 x 5μ
4–septate, about 4.5 per cent, 39 x 5.26μ
5–septate, about 2 per cent, 55 x 5.5μ
6– and 7–septate, about 0.5 per cent, 58 x 5.6μ

Appel and Wollenweber's measurements for 3–septate conidia average 36 x 5.25 (31–40 x 4.5–5.5)μ

RAMULARIA (Unger) Fries (Fig. 1, a to c)

Unger, F., Exantheme der Pflanzen, page 169. 1833. Fries, E. M., Summa vegetabilium Scandinaviae, page 493. 1849. Cf. Wollenweber, H. W., Phytopath. **3**:33. 1913. Wollenweber, H. W., Phytopath. **3**:207–211. 1913.

Differs from Fusarium mainly by nearly cylindrical, apedicellate conidia, with rounded apex; plectenchymic stromata flat to well-developed, wartlike, short column-like structures typically present; conidia borne on conidiophores on aërial mycelium, or on plectenchymic substratum; microconidia (that is, a distinct, abbreviated type of conidia) absent.

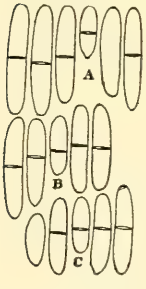

Fig. 49.—*Ramularia eudidyma.* a, *Conidia from aërial mycelium from 55-days-old culture on red raspberry cane plug;* b, *sporodochial conidia from 153-days-old culture on hard lima-bean agar;* c, *conidia from aërial mycelium from 25-days-old culture on slightly acidified hard potato agar*

1. *Ramularia eudidyma* Wr. (Figs. 1b and 49)

Wollenweber, H. W., Phytopath. **3**:221–222, Pl. xxi, fig. c. 1913.

Syn. *Fusisporium didymum* Hartig. 1846.
Fusarium didymum (Hart.) Lindau. 1909.
Fusarium didymum (Hart.) Ap. et Wr. 1910.
Ramularia didyma (Hart.) Wr. 1913.

Conidia nearly cylindrical, with both ends rounded or basal ends sometimes papillate, mostly 1–septate, 23 x 4.87 (21–26 x 4.7–5)μ;[58] 0– to 2–septate conidia also found, 3–septate very rare; chlamydospores mostly intercalary, 8–11μ in diameter; color of spore mass, from white to yellowish; color of plectenchyma, dense brown.

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[58] According to Wollenweber's data (1913 c: 234) the average size of 1–septate conidia is 26.4 x 5 (21–29 x 4.25–5.5)μ.

Hab. On decaying tubers of *Solanum tuberosum* and on roots of *Rubus idæus,* also in soil, Europe.

The organism was not isolated by the writer, but was briefly studied from a culture received through the courtesy of Dr. Wollenweber.

2. *Ramularia Magnusiana* (Sacc.) Lindau (Figs. 1C and 50)

Cf. Wollenweber, H. W., Phytopath. 3:221, 234; Pls. XX, figs. F to H, and XXI, fig. A. 1913.

Conidia of the same type as those of *R. eudidyma,* also mostly 1–septate, 23.6 x 4.3 (18–27 x 3.5–5)μ;[59] 0– to 3–septate conidia also occurring, not numerous, sometimes catenulate; plectenchyma from olive to coffee brown. Much like *R. candida* (Ehr.) Wr., differing from it by old rose color on rice and by thinner conidia sometimes borne in chains. 0–septate chlamydospores 10 x 11 (7–11 x 9–16)μ; 1–, 2–, and pluri–septate chlamydospores also occurring.

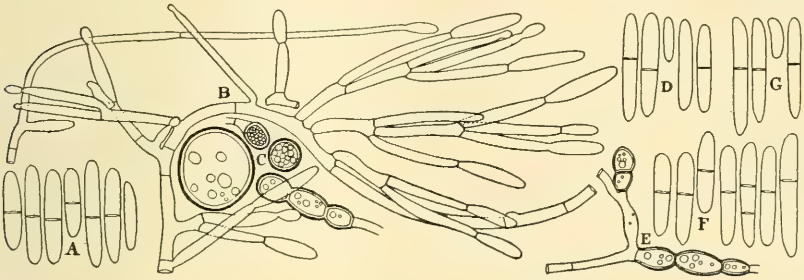

FIG. 50.— *Ramularia Magnusiana.* A, *Conidia from aërial mycelium,* B, *conidiophores, from 11-days-old culture in a petri dish on neutral hard potato agar;* C, *chlamydospores,* D, *sporodochial conidia, from 51-days-old culture on red raspbery cane plug;* E, *chlamydospores from 11-days-old culture in a petri dish on neutral hard potato agar;* F, *conidia from aërial mycelium from 22-days-old culture on slightly acidified hard potato agar;* G, *sporodochial conidia from 153-days-old culture on hard lima-bean agar with 2 per cent glucose*

Hab. Common on tubers of *Solanum tuberosum,* also found on the leaves of Trientalis and on the roots of Acer, in Europe and America.

The fungus was isolated several times from rotted potato tubers, New York, the tubers being covered with from a few to many, from dark brown to brick red, plectenchymic bodies, from 2 to 3 millimeters in diameter and from 1 to 4 millimeters high, often bearing characteristic, almost cylindrical, usually 1–septate, conidia.

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[59] The size is after Wollenweber (1913 c: 234).

The strains isolated by the writer differ from those described by Wollenweber, by more reddish color of plectenchyma, by somewhat narrower conidia and larger (?)[60] chlamydospores, and by the absence of persistent conidial chains.

Measurements of conidia of strain 63 on a few different media are as follows:

On slightly acidified hard potato agar, culture twenty-two days old; conidia from aërial mycelium:

Conidia: 0–septate, rare
1–septate, 95 per cent, 26.5 x 4.1 (18–30 x 3.5–4.7)μ
2–septate, 5 per cent, about the same size as 1–septate

On red raspberry cane plug, culture fifty-one days old; conidia from a sporodochium:

Conidia: 0–septate, 50 per cent, 13 x 3 (8–28 x 2.6–4)μ
1–septate, 50 per cent, 27 x 3.9 (24–31 x 3–4.3)μ

On hard lima-bean agar, culture one hundred and fifty-three days old; conidia from a sporodochium:

Conidia: 0–septate, 1 per cent, 10 x 3.4 (7.9–12.3 x 3–3.5)μ
1–septate, 99 per cent, 26 x 3.8 (20–32 x 3.5–4)μ

Average of the above measurements:

Conidia: 0–septate, 17 per cent, 11.5 x 3.9μ
1–septate, 81 per cent, 26.5 x 3.9μ
2–septate, 0 to 5 per cent, same as 1–septate

The average size of conidia according to Wollenweber's measurements is:

Conidia: 0–septate, rare, 14 x 3.8μ
1–septate, 100 per cent, 23.6 x 4.3μ
3–septate, rare, 30 x 5μ

It is possible that strain 63, on a thorough comparative study of it with *R. Magnusiana*, may prove to be a distinct variety; but it is evident that the resemblance between the two in all important characters is close enough to warrant its being placed in the species *R. Magnusiana*.

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[60] Wollenweber does not give the size of chlamydospores, but it can be interpreted that it is the same as for *R. candida* (Ehr.) Wr., namely, 5–8μ in diameter. Chlamydospores of the strains described here on corn meal agar in cultures one hundred and seventy-three days old measured 10 x 11 (7–11 x 9–16)μ.

3. **Ramularia Solani** n. sp. (Figs. 1A and 51)

Conidia from nearly cylindrical to slightly curved, with both ends rounded or with basal cell slightly papillate, mostly 1– and 2–septate; 1–septate measuring 29.5 x 6.5 (28–30 x 6.2–6.7)μ, and 2–septate measuring 32 x 6.4 (26–33.7 x 6.3–6.8)μ; plectenchyma flat, usually chocolate brown; aërial mycelium from 2 to 4 millimeters high, from medium dense to medium loose, from chamois to deep olive-buff, on potato agar rich in glucose, often with a chocolate-drab tinge; substratum, on the same medium, in zones of from sepia to tawny olive, and in old cultures to Saccardo's amber; conidia in mass from nearly white to deep olive-buff; no chlamydospores observed.

Hab. On rotted tubers of *Solanum tuberosum*, New York.

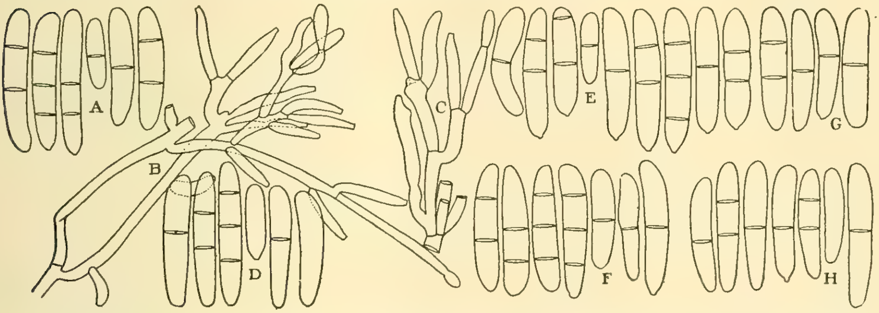

Fig. 51.— *Ramularia Solani.* A, *Sporodochial conidia from 12-days-old culture on oats;* B *and* C, *conidiophores from 34-days-old culture on hard lima-bean agar;* D, *conidia from aërial mycelium from 25-days-old culture on slightly acidified hard potato agar;* E, *conidia from aërial mycelium from 34-days-old culture on hard lima-bean agar;* F, *sporodochial conidia from 8-days-old culture on hard lima-bean agar;* G, *sporodochial conidia from 55-days-old red raspberry cane plug;* H, *sporodochial conidia from 67-days-old flask culture on hard potato agar with 2 per cent glucose*

Latin description.—Conidiis ferme cylindricis vel parum curvatis, utrisque finibus rotundatis vel cella infima parum papillata, plerumque 1–2–septatis; conidiis 1–septatis, 29.5 x 6.5 (28–30 x 6.2–6.7)μ, vel 2–septatis, 32 x 6.4 (26–33.7 x 6.3–6.8)μ; plectenchymate plano, plerumque "chocolate brown" (R); aerio mycelio 2–4 mm. alt., e mediocriter denso mediocriter laxo, e "chamois" (R) "olive-buff" (R), saepe in agare Solani tuberosi perglucoso "chocolate-drab" (R) tincto; substrato, eodem in agare, e "sepia" (R) "tawny olive" (R) in zonis, vel in culturis maturis

"Saccardo's amber" (R); conidiis in totum e ferme albo olivaceo-gilvis; nullis chlamydosporis.

Hab. In tuberibus putridis Solani tuberosi, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture twenty-five days old; conidia from a sporodochium:

Conidia: 0–septate, rare
1–septate, 25 per cent, 28–35 x 5.5–6.3μ
2–septate, 45 per cent, 26 x 6.3 (29–39 x 6.7)μ
3–septate, 30 per cent, 37 x 6.5 (30–41 x 6.3–7)μ

On red raspberry cane plug, culture fifty-five days old; conidia from a sporodochium:

Conidia: 1–septate, 63 per cent, 30 x 6.7 (20–34 x 5.5–7.4)μ
2–septate, 37 per cent, 33 x 6.8 (25–37 x 6.5–7.4)μ

On the same medium as above, culture one hundred and thirty-six days old; conidia from a sporodochium:

Conidia: 0–septate, few, 19 x 5.4μ (only a few measured)
1–septate, 30 per cent, 28 x 6.2 (20–32 x 5.2–6.8)μ
2–septate, 62 per cent, 33 x 6.5 (28–40 x 6–7)μ
3–septate, 8 per cent, 35 x 6.5 (31–40 x 6–7)μ

On hard lima-bean agar, culture eight days old; conidia from a sporodochium:

Conidia: 1–septate, 44 per cent, 30 x 6.3 (22–35 x 5.5–6.5)μ
2–septate, 49 per cent, 33.7 x 6.4 (29–39 x 6–6.6)μ
3–septate, 7 per cent, 36 x 6.5 (31–40 x 6–6.6)μ

On same medium and from same sporodochium as above, culture thirty-four days old:

Conidia: 0–septate, rare
1–septate, 46 per cent, 30 x 6.2 (23–37 x 5.4–6.5)μ
2–septate, 35 per cent, 33.6 x 6.3 (24–39 x 5.8–6.5)μ
3–septate, 19 per cent, 36.9 x 6.5 (32–39 x 6.1–7)μ

Average of the above measurements:

Conidia: 0–septate, rare, 19 x 5.4μ
1–septate, 42 per cent, 29.5 x 6.5μ
2–septate, 45 per cent, 32 x 6.4μ
3–septate, 13 per cent, 36 x 6.5μ

This organism, together with *Fusarium udum* var. *Solani*, was isolated by the writer only once, from a planting of diseased tissues of a potato tuber affected with a superficial dry rot, received from Long Island, New York.

By the shape and size of its conidia it very closely resembles *R. macrospora* Fres. (see Wollenweber 1913 c:222–223, 235, Pl. XX, A and B, and Pl. XXI, E), but differs mainly by the absence of oval, continuous conidia on aërial mycelium, and by the absence of chlamydospores.[61]

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[61] Chlamydospores were searched for very carefully in cultures on different media and of different ages, very old cultures being included, but were never observed. According to Wollenweber (1913 c: 208) such organisms, Ramularia-like but without chlamydospores, produce perfect forms of the genus Mycosphærella; although the organism described here under the name of *R. Solani*, when grown on various media, did not produce chlamydospores, nevertheless it has been retained in this genus because of its otherwise perfect resemblance to the other unquestionable species of Ramularia.

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Submitted for publication February 10, 1915.

EXPLANATION OF PLATES

All the plates were reproduced by three-color process from living cultures. The cultures were in all cases, unless otherwise specified, forty days old when photographed, and were grown at laboratory room temperature ranging from 20° to 25° C.

Cultures of Plates I to IV were, with a few specified exceptions, grown on hard potato agar with 10 per cent glucose, and were kept in a strong diffuse daylight and in air nearly saturated with moisture.

All cultures of Plates V to VII were on sterilized potato tuber plugs in test tubes which were kept at the above temperature but in very weak diffuse light.

PLATE I. FUSARIA OF POTATOES

Fig. 1. Fusarium Solani, from above
Fig. 2. F. striatum, from above
Figs. 3 and 4. F. Martii var. minus: 3, on acidified medium from below; 4, on hard oat agar from above
Figs. 5 and 6. F. coeruleum, from above and below
Figs. 7 and 8. F. oxysporum var. resupinatum, from above and below
Figs. 9 and 10. F. lutulatum var. zonatum, from above and below
Figs. 11 and 12. F. sclerotioides, in dark, from above and below

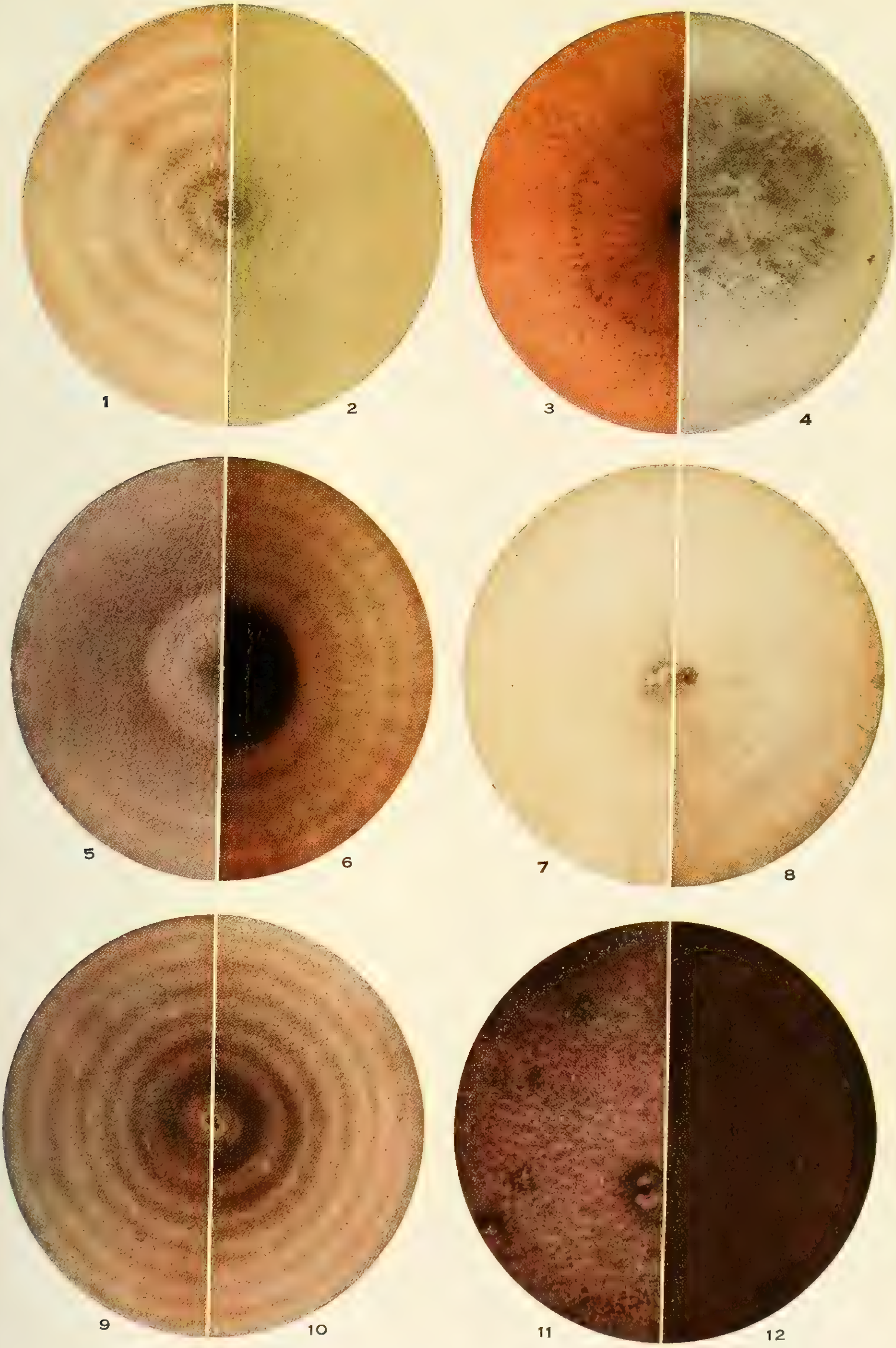

PLATE I—FUSARIA OF POTATOES

PLATE II. FUSARIA OF POTATOES

Figs. 1 and 2. Fusarium sclerotioides var. brevius, from above and below; 1, culture fifteen days old
Figs. 3 and 4. F. redolens var. Solani, from above and below
Figs. 5 and 6. F. lutulatum, from above and below; 6, culture on slightly acidified medium fifteen days old
Figs. 7 and 8. F. arcuosporum, from above and below
Figs. 9 and 10. F. lucidum, from above and below; fig. 9, culture fifteen days old, in dark
Fig. 11. F. subulatum, from above; hard oat agar
Fig. 12. F. subulatum var. brevius, from below, on slightly acidified medium

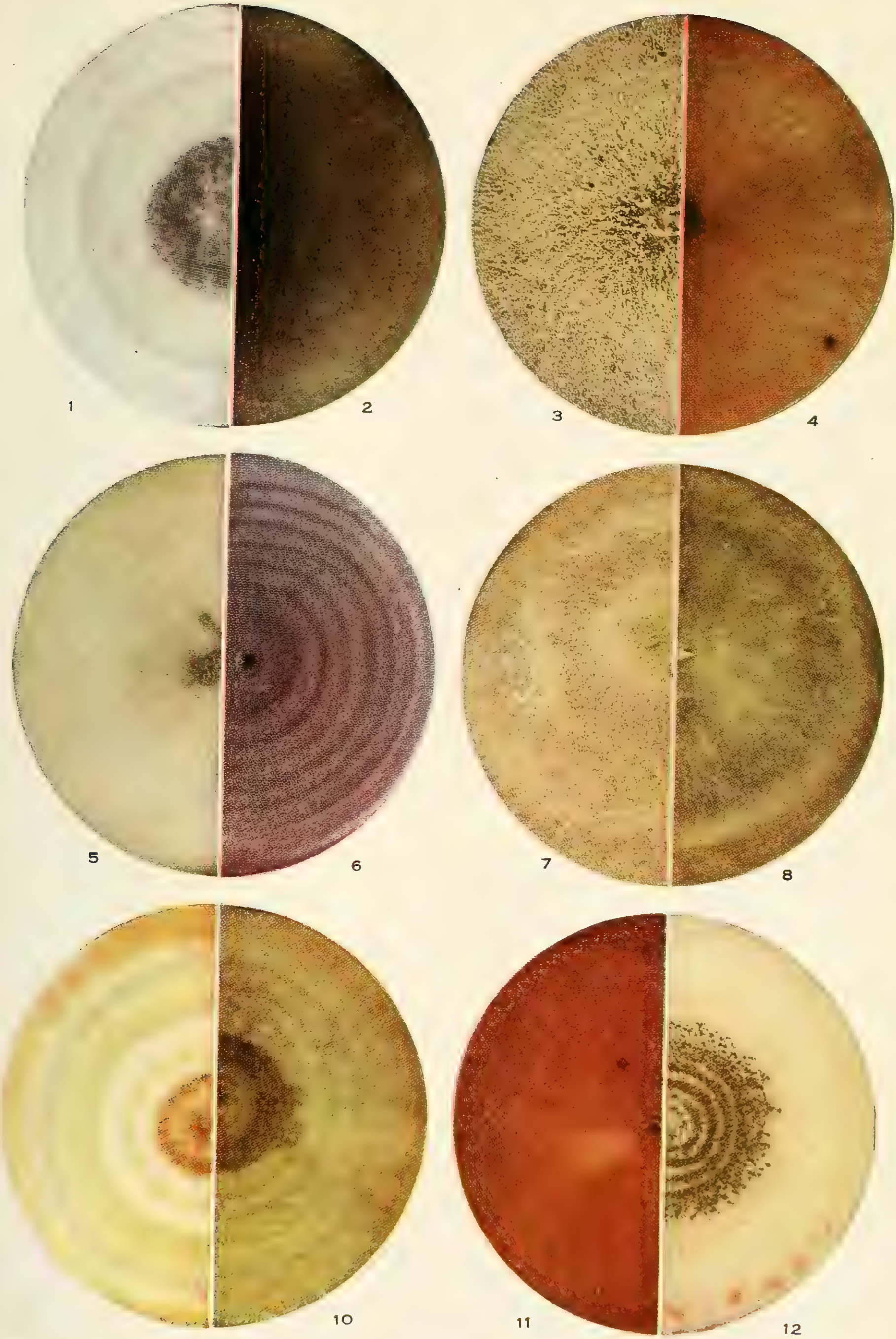

PLATE II – FUSARIA OF POTATOES

PLATE III. FUSARIA OF POTATOES

Fig. 1. Fusarium sporotrichioides, from above; in dark
Fig. 2. F. bullatum var. roseum, from above
Figs. 3 and 4. F. arthrosporioides, from above and below; in dark
Figs. 5 and 6. F. sanguineum var. pallidium, from above and below
Figs. 7 and 8. F. sanguineum, from above and below; in dark
Figs. 9 and 10. F. ferruginosum, from above and below
Figs. 11 and 12. F. clavatum, from above and below

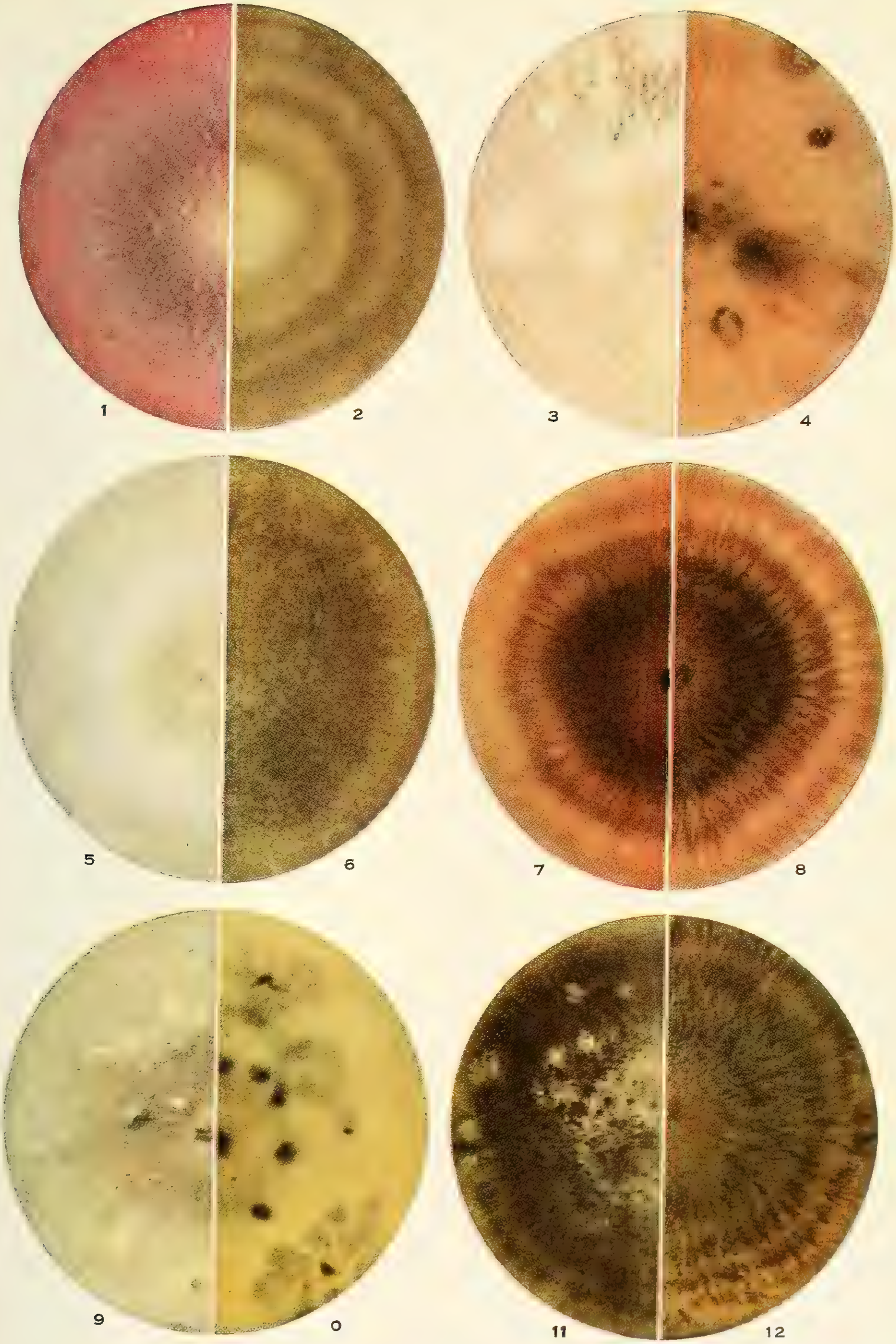

PLATE III—FUSARIA OF POTATOES

PLATE IV. FUSARIA OF POTATOES

Figs. 1 and 2. Fusarium culmorum var. leteius, from above and below
Fig. 3. F. subpallidum var. roseum, from above; on slightly acidified medium
Fig. 4. F. discolor, from below
Figs. 5 and 6. F. discolor var. triseptatum: 5, from above, in dark on neutral medium; 6, from above, in light on slightly acidified medium
Fig. 7. F. caudatum var. Solani, from above, neutral medium
Fig. 8. F. trichothecioides, from above
Fig. 9. F. culmorum from above, on hard oat agar
Fig. 10. F. culmorum var. leteius, from above, on hard oat agar
Fig. 11. F. discolor var. sulphureum, from above
Fig. 12. F. lucidum, from above, slightly acidified medium, fifteen days old

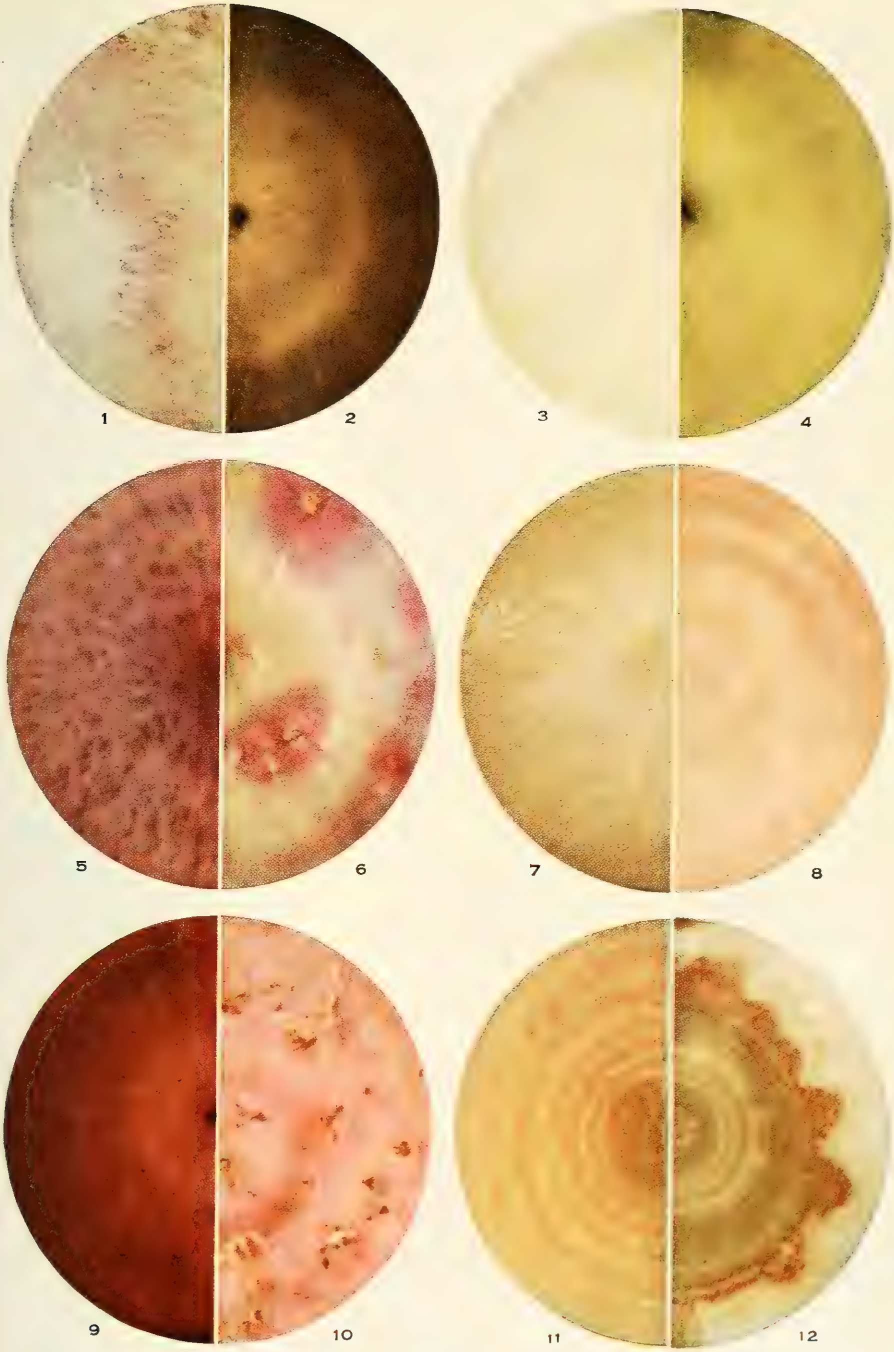

PLATE IV—FUSARIA OF POTATOES

PLATE V. FUSARIA OF POTATOES

Fig. 1. Fusarium sclerotioides
Fig. 2. F. redolens var. Solani
Fig. 3. F. lutulatum
Fig. 4. F. lutulatum var. zonatum
Fig. 5. F. oxysporum var. resupinatum
Fig. 6. F. oxysporum
Fig. 7. F. oxysporum var. asclerotium
Fig. 8. F. culmorum
Fig. 9. F. culmorum var. leteius
Fig. 10. F. discolor var. triseptatum
Fig. 11. F. discolor
Fig. 12. F. subpallidum

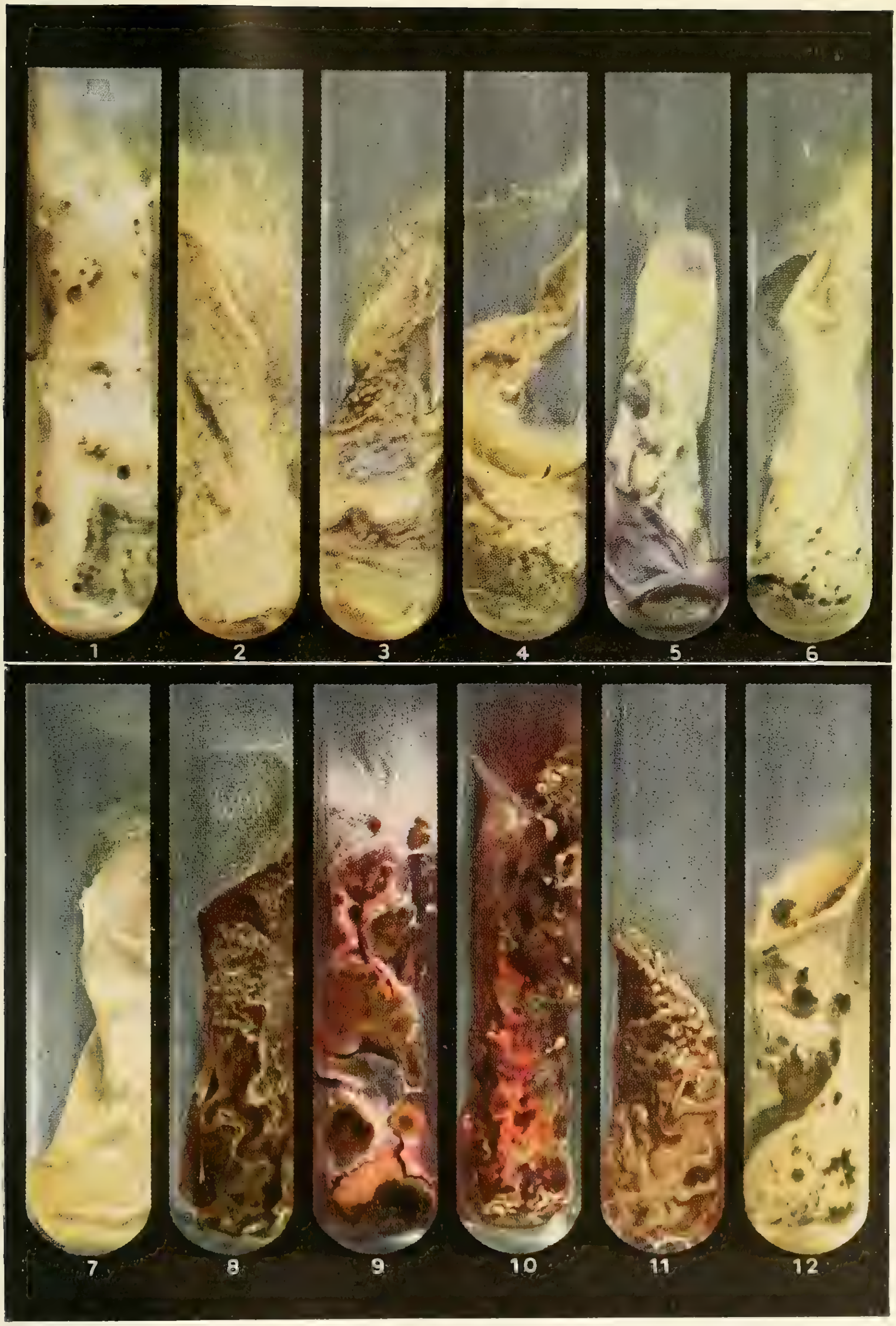

PLATE V—FUSARIA OF POTATOES

PLATE VI. FUSARIA OF POTATOES

Fig. 1. Fusarium sanguineum
Fig. 2. F. ferruginosum
Fig. 3. F. caudatum var. Solani
Fig. 4. F. coeruleum
Fig. 5. F. Martii var. viride
Fig. 6. F. Martii var. minus
Fig. 7. F. Solani
Fig. 8. F. radicicola
Fig. 9. F. anguioides var. caudatum
Fig. 10. F. arcuosporum
Fig. 11. F. anguioides
Fig. 12. F. lucidum

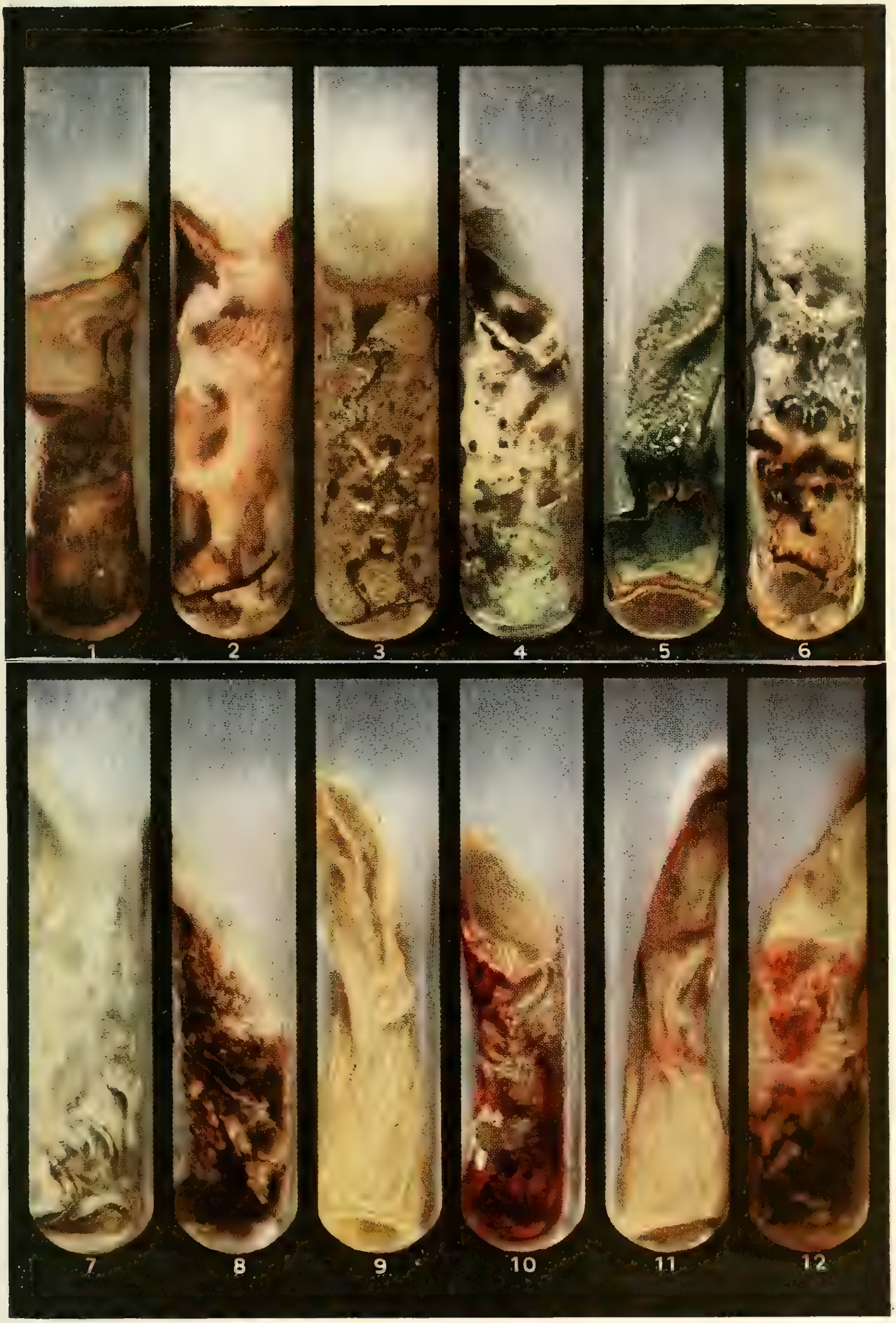

PLATE VI—FUSARIA OF POTATOES

PLATE VII. FUSARIA OF POTATOES

Fig. 1. Fusarium truncatum
Fig. 2. F. clavatum
Fig. 3. F. subulatum var. brevius
Fig. 4. F. subulatum
Fig. 5. F. metacroum
Fig. 6. F. effusum
Fig. 7. F. sanguineum var. pallidium
Fig. 8. F. falcatum var. fuscum
Fig. 9. F. arthrosporioides var. asporotrichius
Fig. 10. F. biforme
Fig. 11. F. arthrosporioides
Fig. 12. F. diversisporum, pseudopionnotal stage

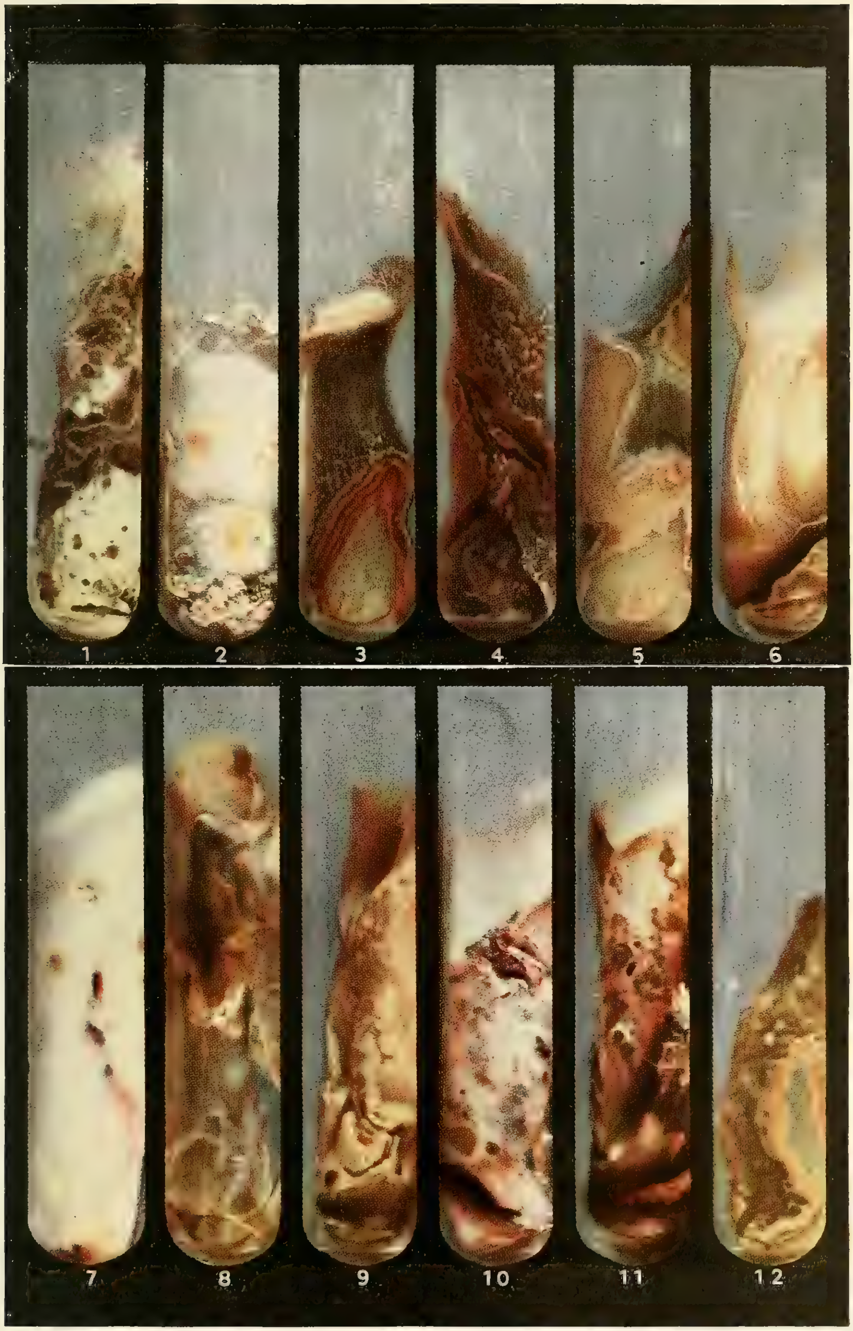

PLATE VII—FUSARIA OF POTATOES

JUNE, 1915 MEMOIR No. 7

CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

SENILE CHANGES IN LEAVES OF VITIS VULPINA L. AND CERTAIN OTHER PLANTS

By HARRIS M. BENEDICT

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SENILE CHANGES IN LEAVES OF VITIS VULPINA L. AND CERTAIN OTHER PLANTS

SENILE CHANGES IN LEAVES OF VITIS VULPINA L. AND CERTAIN OTHER PLANTS[1]

Harris M. Benedict

The inevitable onset of senile deterioration after a relatively short period of activity is the basic tragedy of life. And yet, in comparison with the amount of research that has been directed toward the discovery of the causes of living activities, very little has been done to determine the causes of the approach of senility. Biologists seem to have tacitly agreed to find out what causes the living machine to run before they seek the reasons why it stops. This attitude has been further encouraged by the belief that the causes of senility could not be understood until the laws governing living action were determined.

The work that has been done is naturally of two kinds — attempts to determine what changes of function and structure are characteristic of the increasing age of an organism, and the promulgation of various theories as to the causes of senility. So much of the work on senility has been carried on with animal organisms that this field in plant life may be said to be unentered; the reasons for the neglect of plants in this respect will be dealt with later. It seems advisable, therefore, as the only available basis for approaching this problem in plants, to prepare a brief summation of the results of the investigations on senility in man and in the lower animals.

SENILE CHANGES IN ANIMALS

The chief difficulty in the search for the changes due to senility is the constant presence in the living organism of modifications of tissue and function produced by unfavorable conditions other than age, both internal and external. Much of the work on record has been marred by inadequate appreciation of this fact. Furthermore, the older the organism becomes, the greater is the probability that effects of these other adverse influences will be present. It follows, therefore, that the condition of extreme senility, which has been commonly studied, is actually the most difficult stage to investigate for changes due to age alone. Those theories of

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[1] Laboratory of Plant Physiology, Cornell University. Contribution No. 15.

senility that are more or less fanciful have been based on conditions found in extremely senile animals.

It is now generally recognized that the forces causing senility and natural death are not dormant in early life and later roused to action, but instead are at work from the time when embryonic development begins until death occurs, and, in fact, as Minot (1908)[2] has so efficiently emphasized, are most active at the beginning. Therefore, in view of the difficulty involved in the investigation of extremely senile tissue and of the constant presence of senile changes throughout life, the importance of comparison between younger stages is manifest; and in the accompanying summary of the results of investigations, first rank is given to those characteristics that can be shown to undergo continuous change from the beginning. The results of the various essential researches into senility in animals are here classified under three headings: (1) Senile changes in physiological activities; (2) Senile changes in the structure of animal organs; (3) Senile changes in the structure of animal cells. It is evident that in this classification there will be some overlapping, but its advantages in permitting a concise and clear-cut arrangement of the various results of different investigators outweigh this defect.

This very condensed summary of the results of investigations on animal senility gives at least a conception of the difficulties involved. Evidently, however, a start has been made in the various avenues of approach to the problem.

SENILE CHANGES IN PHYSIOLOGICAL ACTIVITIES

Deterioration evident from the beginning

Decrease in rate of growth.— The most obvious characteristic of senility is a decrease in rate of growth. The rate of decrease found in animals thus far investigated may be illustrated by figures obtained from the study of human development. Hertwig (1906) estimates that the human ovum has a volume of 0.004 cubic millimeter, while the size of the child at birth is 4,000,000 cubic millimeters — an increase of 100,000,000,000 per cent in nine months. According to Minot (1908) the gain in weight during the same period is 500,000,000 per cent. It is evident that the rate of growth of the embryo is extraordinary. Mühlmann (1900 and 1901) states that the rate of growth of the embryo is greater during the first month than the second, indicating that a decrease in the rapidity of growth sets in even before birth.

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[2] Dates in parenthesis refer to bibliography, page 365.

Data are abundant regarding the yearly percentage of gain in weight made by children. The average increase in weight during the first year, relative to the weight at birth, shows a percentage of increase of 200; that of the second year only from 22 to 30; that of the third year from 14 to 20. From the fourth year to the twenty-fifth the rate of increase is subject to irregular variations, mostly downward. From the twenty-fifth year to the fiftieth there is little or no change in weight, on the average. After fifty years, as a rule, there is a loss in weight. Detailed tables, with graphs showing the steady decrease in rate of growth with increasing age, can be found in Donaldson's papers (1895, 1906, and 1908); while Minot gives carefully selected data from various sources, to which he has added graphic illustrations.

It is evident that the rate of growth in animals thus far investigated declines from the very beginning of individual life, and that this is a manifestation of inherent forces which tend to slow vital activities.

Decrease in rate of respiration.— The rate of respiration in mammalian animals can be investigated only in the years after birth. There is then evident a gradual decrease of the same general type as that found for growth. In the last stage of life a slight increase occurs, similar to that shown in the heartbeat and explained under that topic.

Decrease in rapidity of heartbeat.— The following figures are taken from a compilation by W. T. Porter, quoted in Minot's book, and refer to man:

Age	Mean frequency of heartbeat per minute
Fetus	140
1st month	134
5th year	103
10th year	91
15th year	82
20th year	74
25th year	72
55th year	72
65th year	73
80th year	79

A curve graphically representing the change in pulse rate has been plotted and is shown in figure 52. Here again there is seen a more rapid decrease in early life than later. It is evident that the rates of growth, of respiration, and of heartbeat are but the most obvious indicators of the degree of metabolic activity of the animal protoplasm.

The increase in rate of respiration and of heartbeat that occurs in the last years of life is an example of a struggle between the depressing force of senility and the accelerating stimuli exerted on the lungs and the heart by the other organs of the body. The organs composing the body of an animal are so interrelated that a need in one produces a stimulus to action in any organ which can satisfy that need. In the last years of life the organs supplied with oxygen and relieved of harmful waste products by the failing lungs and heart, reach such a condition that the resulting stimuli become so powerful as to more than neutralize for a time the effects of senility in slowing the beat.

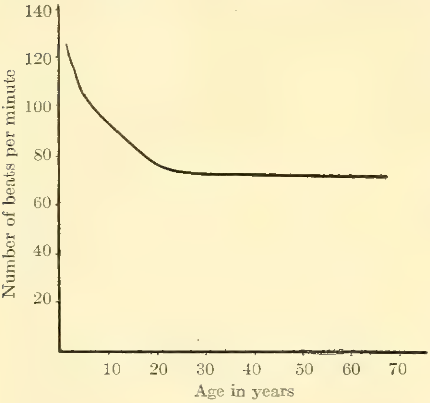

FIG. 52.—*Curve of senile decrease in rate of heartbeat of man*

This power of one part of the body to stimulate another organ leads to many cases of apparent suppression of the effects of senility, especially in the stages of youth and maturity of the animal body. Contrary to the usual custom, these have been placed in a group by themselves as those evidences of senility that become visible only at some stage later than that of youth. These activities are spurred on by the stimuli resulting from their interaction with the other activities of the body. For example, the increase of muscular strength and coordination due to the interaction of the muscular and nervous systems, which characterizes the first third of human life, is made possible.

Deterioration appearing later in life

The first four types of deterioration given as appearing later in life deal with activities in contact with the external world, and are therefore the most obvious.

Deterioration in rapidity and coordination of movement.— According to Mühlmann (1901) the musculature of man ceases to grow at about the age of thirty-six, but the experience of highly trained athletes indicates a considerably earlier appearance of decrease in muscular power and coordination.

Deterioration in the powers of special sense.— The tendency toward deafness and failing sight are common manifestations of deterioration in the powers of special sense.

Deterioration in efficiency of excretion.— While the belief that deterioration in efficiency of excretion is always present is based partly on theoretical considerations rather than on extensive tests, there can be little doubt of its truth. In dealing with the various theories of senility this function will be again referred to.

Deterioration in reproductive power.— Decreased reproductive power is more marked in the female than in the male.

Deterioration in all the internal activities characteristic of living animals.— There can be little question of the presence of a general deterioration in internal activities of animals, even in the central nervous system, but exact investigations are wanting.

SENILE CHANGES IN THE STRUCTURE OF ANIMAL ORGANS

The changes discussed below seem to be of a type not appearing in early life.

Decrease in the size of all organs, due to atrophy of the cells and of the intercellular substance.— Douaud (1867), Kölliker (1897), and others since their times, have investigated the senile atrophy in muscles. Metchnikoff (1908) presents further studies in which he claims that the destruction of the contractile power is produced by a special kind of phagocyte. Senile atrophy of the brain has been investigated in man and in bees by Hodge (1895), who found that the decrease in size was largely due to actual death and disappearance of cells, as well as to shrinkage of the living cells. In all cases when the organs of animals in the condition of extreme senility have been studied, the appearance of loss and degeneration of cells is presented. The ductless glands — the thyroid, for example — show this degeneration and have been utilized by Lorand in the formulation of a theory of senility.

Hardening and thickening of the lining of vessels, lungs, and digestive system, and hardening of cartilage.— Arterial sclerosis has long been associated with senility, even to the extent of being considered a cause (Demange, 1886). The hardening and thickening of all lining membranes is believed to accompany old age, but the experiments have been too few to decide the matter. There seems little question, however, but that such absorbing and excreting surfaces become more rigid with age, and probably less efficient. The hardening of the cartilages, with consequent decrease in the flexibility of the joints and the forward curving of the spine, is universal in man.

Increasing weakness and brittleness of bone.— While other tissues are gaining an undue amount of calcareous material, the strengthening lamellæ of the interior parts of the bone are disappearing and in consequence the bone becomes weaker. The remainder of the bone increases in its calcareous content and decreases in its living substance, thus becoming more brittle as well as weaker.

Tendency of connective tissue to encroach on the higher tissues.— The increasing tendency in old age for injured cells of nerves, muscles, or other highly specialized tissues to be replaced by connective tissue, which is the least highly specialized tissue, has been explained in a number of ways. The generally accepted view is that given by Hertwig (1906). He pictures a single-celled organism as carrying on the functions necessary to itself alone, and thus being acted on only by cytological laws. The cell in a multicellular organism, on the other hand, is subject to both the cytological laws required for its own nourishment and to the organotypical laws governing those of its activities that nerve the whole organism of which it is a part. Hertwig believes that the higher the differentiation of a tissue, the more complete is its obedience to the organotypical laws. As age increases, the organotypical laws weaken, and the lowest tissue, which is least subject to them, is the first to throw off the yoke and attack the surrounding organs. This view is interesting, but purely theoretical.

While the discussion of these four classes of change in organs seems a very inadequate treatment of the effect of senility on animal organs, it is not an unfair expression of the present state of knowledge of these changes. Other changes have been mentioned, but there is even less evidence for them than for those here given.

SENILE CHANGES IN THE STRUCTURE OF ANIMAL CELLS

All the preceding changes must of course be merely magnified outgrowths of changes occurring in the cell. Considering the investigations made as to the effect of senility on the cell itself, it may be seen that very little has been done toward an actual understanding of this problem.

Changes evident from the beginning

Decrease in size of nucleus.— Hodge (1895) found the nuclei of the spinal ganglia to be smaller in the senile than in the young condition. Hertwig (1904 and 1906), however, regards the extreme size of the nucleus characteristic of certain protozoan stages and of the animal ovum as evidence of dormancy rather than of rejuvenescence. Minot gives the interesting evidence that while investigators of the embryonic development of animal eggs make no statement regarding the fact, their drawings invariably show a rapid decrease in the size of the nucleus.

Increase of cytoplasm in amount and in differentiation.— That cytoplasm increases in amount and in differentiation was first emphasized by Minot (1891), who considers the fact of prime importance. Eycleshymer (1904) gives the following data regarding the proportion of cytoplasm to nucleus at different ages of Necturus muscle:

Length of body......	8 mm.	17 mm.	26 mm.	Adult, 230 mm.
Proportion of cytoplasm to nucleus....	2,737–1	4,318–1	8,473–1	23,379–1

The great change in the structure of the cytoplasm by which the highly specialized cells are developed from the undifferentiated cells or the morula stage, is evident.

Changes appearing later

Nucleus irregular in form, and staining like cytoplasm.— Irregularity in form of the senile nucleus, and its staining like cytoplasm, was observed by Hodge (1895) in the spinal ganglia of man.

Local degenerations in cytoplasm.— Cytoplasm with many local degenerations, indicating that portions were undergoing various forms of degeneration, is described for ganglion cells by Hodge (1895). Various forms of fatty degeneration are found in senility in cartilage and in other body cells, indicating loss of power of assimilation or inefficient katabolism.

Accumulation of pigment in the cytoplasm.— Hodge (1895) found a difference in accumulation of pigment in the cytoplasm between young and old cells of the spinal ganglion of man. It has been stated that pigments are excretory masses and that they increase with age.

Deterioration of nucleolus.— The nucleolus diminishes in mass and in staining power. It was Hodge also who first pointed out this fact. Hertwig (1904) emphasized the relation between size of nucleolus and cell activity.

INVESTIGATIONS ON SENILITY IN PLANTS: GENERAL

REVIEW OF LITERATURE

A review of the changes that have been discovered in perennial plants as a result of senility shows that no investigations comparable to the ones above cited have been made. Such statements as are commonly found regarding the effect of age are based on casual observations rather than on investigations. The reason for this seems to be the tacitly accepted belief that, since new leaves and twigs and roots are constantly being formed from persisting embryonic cells, senility as present in animals is out of the question, and the term as used for plants means merely that conditions have become so unfavorable that the growing parts are killed.

This view has been further encouraged by the very great age attained by certain trees, as, for example, the sequoias, the cypresses of Mexico, and the baobab of Cape Verde — the last named estimated by Adanson to be over five thousand years old. Therefore, the idea that trees have the potential power to live forever if the conditions due to their increasing size — injury from winds, greater distance from the soil, and other harmful effects — could be prevented, is not so much formally accepted as passively assumed.

Such statements as have been published are almost entirely from the pens of fruit growers. Among the first of which the writer has found record are the papers by Thomas Andrew Knight, the justly famous English horticulturist, to which reference will be made later. Zorn (1890), nearly one hundred years later, gives about the same list of effects of age, and his list in its conclusion does not fall far short of present knowledge. He says that in old age fruit trees bear less, are later in maturing their fruit, and are more likely to be attacked by scale and disease. He states further, regarding pear trees, that the epidermis is likely to blister, that the trees are less able to withstand cold, and that the fruit is small, deformed, unpalatable, and with more hard flecks in the flesh.

The botanist and the horticulturist of to-day add nothing to Zorn's list. The question whether true senility occurs in perennial plants, therefore, is still an open one. The changes spoken of by Zorn and by more recent writers may be effects of disease of external origin or the result of increase in size of the tree, not real results of senility. De Candolle (1876) investigated the question whether the time of leafing was related to the age of the tree, and decided that the age was negligible compared to the climatic conditions. His results in this one particular seem to have been much more widely applied than he probably intended.

IMPORTANCE AND SIGNIFICANCE OF THE PROBLEM

The importance of determining whether there is any real senile change in plants lies not alone in the scientific need for such knowledge, but also in its direct bearing on the long-disputed question regarding the effect of continuous vegetative propagation of seed-producing plants. For if the new growth from which cuttings are made has not been affected by the time that has elapsed since the plant came from the seed, then its tissue is no more senile than seedling tissue; if, however, the meristematic tissue, which has been so actively growing and dividing since it originated in the embryo sac of the parent plant, has itself suffered the senile deterioration that accompanies activity in animal cells, then the tissues arising from this meristematic tissue must partake of its senility.

The processes of division and growth require considerable expenditure of energy; therefore, if the plant cell is not entirely immune to senile deterioration, the meristematic as well as the specialized tissues should undergo such change. In fact, in the ontogeny of the animal, senile deterioration is most rapid at the time when specialization is least, when the only activities of the cells are division and growth. There is no inherent reason, therefore, why meristematic and cambial cells should be put in a different class from that of specialized cells as regards the possibility of senile degeneration.

It may be true — no exact comparisons as to this point are on record — that the meristematic cells have a greater proportion of nucleus to cytoplasm than have the specialized cells which develop from them, and thus, according to the views of Minot and Hertwig, the former would be potentially younger; but that is not the point at issue. The real issue is, provided always that the evidences of senile deterioration found in animal cells may serve as a guide to senility in plant cells, whether the meristematic cells of the older perennial differ from those of the seedling in this or in other respects.

It may be advisable to again refer at this point to an argument which possibly has had some weight; namely, that the very great age attained by certain trees is an indication that they must be immune from senility as this is known in animals. Some of these trees have lived for five thousand years, or many times the age to which man has attained. This argument can be applied to the human being with most interesting and reassuring results. The rotifer *Pleurotricha haffkini*, studied by Metchnikoff (1908), has a total span of life of three days. Applying the above argument, a child who reaches the age of three months, having existed forty times as long as another organism, may be assumed to be immune from the stern decree of senile laws. The elephant lives one hundred thousand times as long as the rotifer; and in general the extraordinary differences in duration of life found in animals in which undoubted senility occurs, robs the fact that plants in general are possibly longer-lived than animals of any significance as regards senility.

INVESTIGATIONS ON SENILE CHANGES IN LEAVES OF VITIS VULPINA L. AND CERTAIN OTHER PLANTS

PLAN OF INVESTIGATION

It would seem that the logical method of attacking the problem is by a careful comparision of the same organ in young and old plants of the same genus for evidences of senile deterioration in structure or function. It was considered desirable to choose a plant that was marked by extreme vigor in putting forth new growth each year, in order to minimize as much as possible the chance of the presence of unfavorable conditions other than age. The wild grape, *Vitis vulpina* L., answered this requirement. Any one who has noticed the long shoots and luxuriant foliage produced by this vine each spring must feel that the conditions for its nutrition and general health are good, and that the harmful results usually ascribed to the increasing size of a plant are not in this case serious.

For several reasons the organ selected was the leaf, not the meristematic tissue itself. In the first place, not only must the presence of any change in the meristematic cells effect changes in the specialized cells produced from them, but the effects must be more evident than the conditions that produced them, for the same reason as that accounting for the fact that any internal differences between two seeds will be displayed much more plainly in the plants that are produced from them than in the seeds themselves. Thus, changes too minute to be perceived in the meristematic cells might produce very obvious differences in the mature leaf. In the second place, the leaves are renewed each year, and therefore, according to the commonly accepted conception, should be entirely immune from any sign of age; or, to express this in another way, any sign of senility found would be of exceptional importance in its bearing on the problem. In the third place, the leaf is more accessible than other organs.

The many careful researches that are on record regarding the effect of all factors excepting age on the structure and functions of the leaf seemed to justify the opinion that if any differences were found it would be possible to decide whether they were due to one of these factors or whether they must be referred to age.

Leaves from young and old vines of *Vitis vulpina* were collected and were subjected to a preliminary examination by means of a hand lens. The most striking difference apparent was observed in the venation. On holding the leaf blades to the light, the meshes formed by the most minute veinlets appeared larger in the leaves of young vines than in those of old ones. A difference so marked that it could be discerned with a hand lens would be capable of utilization in the field and of practical application in orchard and nursery. Therefore an investigation was begun of the size of the meshes in leaves of vines of different ages.

Collection and preparation of material

In order that the effects of differences in exposure to light and wind and of differences in nutrition might be reduced as much as possible, the country in the vicinity of Ithaca, New York, and of Cincinnati, Ohio, was searched for vines of different ages growing near one another under the greatest possible similarity of external conditions. It proved to be more difficult than was expected to find such paired vines, but at last about twenty pairs were found.

These vines were numbered, and their relative ages were obtained by sawing out a thin slice from one side at the base of the stem. For comparative purposes ten leaves were collected from each vine, as well as cuttings and material for permanent mounts. Care was taken in selecting the leaves to get only fully matured, healthy, normal specimens. Since conditions of humidity and temperature are different at different levels above the ground, the leaves were taken from the lower parts of the vines, not above eight feet from the ground. The effect of light exposure has been shown by Schuster (1908) to be a definite one, and care was taken that all selected leaves were such as had full exposure to the sky, of the same character for each of the paired vines.

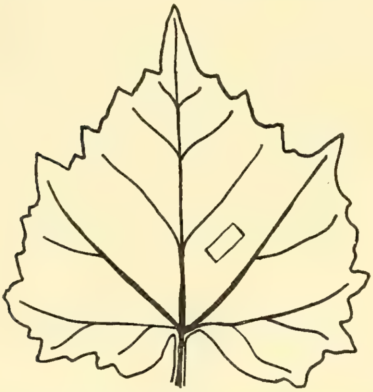

FIG. 53.—*Part of the leaf removed for photographing*

The collected leaves were taken immediately to the laboratory, measured as to length, breadth, and area, and weighed. The venation was then photographed in the following way: A heavy black paper was pasted to a clean glass plate, four by five inches in size. Ten openings, approximately four by ten millimeters in size, were then cut in the black paper. From the same part of each leaf (Fig. 53) pieces a little larger than the openings were cut, and these were laid over the openings, so that each of the ten leaves was represented. A clear glass plate was then laid over all, and the whole was bound together by elastic bands, placed in the negative holder of an enlarging camera, and photographed at an enlargement of three diameters. Negatives showing the veinlets clearly were obtained after some practice, and from these negatives velox prints were made.

The appearance of a leaf, viewed by transmitted light, is as a vast number of aggregations of photosynthetically active cells, occupying the meshes of the network of veinlets. The size of these aggregations seems to the writer to be of more direct physiological significance than the arbitrary standard introduced by Zalenski — the combined length of the veins in one square millimeter of leaf surface — and therefore determinations of the area of the cell aggregates have here been employed.

The average size of these aggregates, which will hereafter be called vein islets, was determined by dividing the area of each of the openings in the paper by the number of vein islets that filled it in the photograph. The counting was done under a lens, and a sharp needle was used to prick each vein islet as it was counted on the photograph.

INFLUENCE OF CERTAIN FACTORS ON VENATION

While the literature on the venation of leaves is very extensive, most of it is concerned with the development, structure, and function of the large veins — the midrib and its main branches. Only one phase of these investigations is directly important to the present problem, and that is the result of the controversy over the question whether the veins follow a course in the leaf entirely predetermined by heredity, or whether external conditions while the leaf is expanding can exert an influence.

The paleontological botanists, as represented by von Buch (1852), went so far as to say that, no matter how much the leaf might expand, no more nerves could arise than those present in the leaf while in the bud. The attack on this idea began with the discovery by von Nägeli (1855) that in the leaves of *Aralia spinosa* the nervature was not completely performed in the bud but arose in part later. Prantl (1883) showed that in many dicotyledons, during the expanding of the leaf, the development of new nerves took place in the direction of the greatest expansion. Deinega (1898), with further evidence obtained from the study of both monocotyledons and dicotyledons, established the fact that the nervature of the leaf is not performed in the bud to any great degree.

It still remained to be proved, however, that external conditions exert an undoubted effect on the nervature as it is laid down in the expanding leaf. While a long list of investigators have determined the effect of light and shade, dryness and moisture, on various structures of the leaf, practically no studies had been made regarding the effects of these external factors on venation until the work of Schuster (1908). It is true that a paper by von Zalenski (1902) was published, in which it was shown that in plants of the same genus a species inhabiting a dry location has a greater development of the finest nerves than another species inhabiting a wet location. Von Zalenski found that *Trifolium lupinaster*, which lives in a dry situation, has a combined length of veins to the square millimeter of leaf blade of 1065 millimeters; while *Trifolium medium*, a moisture-loving plant, has a length of veins of but 460 millimeters. This, however, does not eliminate the effects of heredity. Schuster's investigation, on the other hand, was a detailed study of the effect of definite environmental factors on the same species. His results will be referred to frequently in the course of this paper.

The study of the arrangement of the finest veins of the leaf has the advantage that these veins are directly in contact with the active cells of the leaf, and must therefore be influenced by the state of the activities of these cells. The direct stimuli causing the development of branches from the sides of the existing veins, which thus subdivide the leaf blade, are not yet definitely known, but they must be affected to some degree by the conditions of the cells in the area into which the new branches penetrate. This matter will be taken up later. It is referred to here in order to emphasize the fact that the arrangement of the nerves in the leaf is related to the activities of the other cells of the leaf.

Uniformity in size of vein islets on different parts of a leaf

Schuster (1908) states that there is a definite uniformity as regards venation in all parts of the leaf, except close to the midrib and at the apices. Many tests were made of different parts of the same leaf of *Vitis vulpina* and a variation of less than three per cent was found. One test on a leaf of vine 2 gave the following figures:

	Square millimeters
Average of areas of five pieces from the right side....	0.4560
Average of areas of five pieces from the left side......	0.4567
Largest average of one piece from either side........	0.4613
Smallest average of one piece from either side......	0.4511

In other words, the largest average found was one per cent above the average for the whole leaf, while the smallest was one and one-tenth per cent below, and this may serve as a typical example.

Influence of size of leaf blade on size of vein islets

Schuster's results indicate that the relation between the size of the leaf and the combined length of veins in a given area is not of marked importance. The writer's measurements with *Vitis vulpina* also indicate that the size of the leaf has comparatively little effect. The figures in table 1 may be considered as typical:

TABLE 1. AREA OF VEIN ISLETS IN LEAVES OF DIFFERENT SIZES ON THE SAME PLANT. VINE 29

Number of leaf	Breadth of leaf (centimeters)	Length of leaf (centimeters)	Area of leaf (square centimeters)	Area of vein islets (square millimeters)
1	11	14	108	0.3762
2	17	16	190	0.3663
3	12	12	101	0.3818
4	11	11	85	0.3597
5	13	13	117	0.3669
6	14	14	117	0.3733
7	13	15	127	0.3681
8	15	17	179	0.3703
9	17	21	250	0.3777
10	14	15	147	0.3784

It seems at first thought so natural to suppose that the size of vein islets would be larger in the larger leaves, that a series of leaves was collected from vines ranging in age from seven to eleven years. The size of each leaf and that of its vein islets were measured, and the results are presented in table 2 and graphically represented in figure 54.

It is evident, therefore, that the formation of new veinlets keeps pace with the expansion of the leaf. A most striking example repeatedly found in another plant may be of interest here. Less than ten feet apart stood two trees of *Tilia americana*, one three inches in diameter, the other fourteen inches. The larger tree had been cut down, and from the stump were

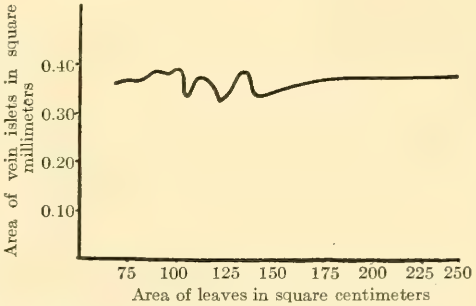

FIG. 54.—*Curve showing that size of leaf does not determine size of vein islets*

TABLE 2. AREA OF VEIN ISLETS IN LEAVES OF DIFFERENT SIZES FROM VITIS VINES WITH FROM SEVEN TO ELEVEN RINGS

Number of leaf	Area of leaf (square centimeters)	Area of vein islets (square millimeters)
1	73	0.3580
2	77	0.3691
3	85	0.3694
4	92	0.3814
5	96	0.3761
6	101	0.3818
7	105	0.3812
8	109	0.3207
9	112	0.3669
10	116	0.3612
11	123	0.3391
12	126	0.3186
13	131	0.3217
14	137	0.3716
15	142	0.3197
16	147	0.3263
17	178	0.3703
18	190	0.3663
19	250	0.3777

growing several thick water sprouts. The relative size of the huge leaves growing on the water shoots and on the small tree was determined, as well as the size of the vein islets, with the following results:

	Area of leaf (square centimeters)	Area of vein islets (square centimeters)
Smaller tree	30	0.04
Water sprouts	1,560	0.02

The larger leaves had the smaller vein islets. The marked decrease in size of the vein islets is due to age. Similar results have been obtained from leaves borne on water sprouts of other varieties of trees.

Influence of thickness of leaf on size of vein islets

While very great differences in thickness of the leaves were not expected to occur, it seemed necessary to determine whether this factor might affect the size of the islets. This was investigated by collecting various leaves from vines of approximately the same age, the variation being a few years, and determining the weight per 100 square millimeters of leaf blade of each leaf and the size of the vein islets of each. The difference in weight is taken to mean differences in thickness, as all the leaves were picked at the same time of day. The data obtained are given in table 3:

TABLE 3. AREA OF VEIN ISLETS IN LEAVES OF DIFFERENT THICKNESSES

Number of leaf	Weight of 100 square millimeters of leaf (grams)	Area of (islets square millimeters)
1	0.83	0.3597
2	0.87	0.3669
3	0.89	0.3663
4	0.91	0.3207
5	0.99	0.3818
6	1.04	0.3777
7	1.16	0.3263
8	1.20	0.3197
9	1.36	0.3217
10	1.43	0.3696
11	1.51	0.3612
12	1.61	0.3667
13	1.63	0.3580
14	1.67	0.3716
15	1.69	0.3691

Influence of such differences in light as are found in nature on the size of vein islets

Schuster found that the effect of light on the veins was not the same in all plants. The leaves of some plants showed an increased amount of venation (smaller vein islets) when grown in the shade, others were not affected, and still others showed a decrease. One of the plants investigated by Schuster was *Ampelopsis veitchii*, a genus of the *Vitaceae*, and this showed, if anything, a tendency to decrease in amount of venation in strong light (resulting in increase in size of vein islets). The same tendency was found to be true in *Vitis vulpina* to a slight degree (table 4).

TABLE 4. EFFECT OF LIGHT AND SHADE ON SIZE OF VEIN ISLETS. VINE 53

Leaves growing in shade (number of leaf)	Area of islets (square millimeters)	Leaves growing in light (number of leaf)	Area of islets (square millimeters)
1	0.1511	1	0.1543
2	0.1376	2	0.1678
3	0.1654	3	0.1751
4	0.1543	4	0.1642
5	0.1517	5	0.1657
6	0.1431	6	0.1635
7	0.1571	7	0.1706
8	0.1635	8	0.1613
9	0.1569	9	0.1534
10	0.1607	10	0.1642
Average	0.1541	....	0.1640

Uniformity in size of vein islets in each vine

Schuster and von Zalenski both treated the length of veinlets as a relatively constant character for each plant and even for each species. Most of their work was done with herbaceous annuals, or with a relatively few garden shrubs or trees which may well have been of nearly uniform age. Unless all the leaves of an individual plant show vein islets of relatively equal size, this character cannot be used as a criterion of the age of the vine. In table 5 are shown conditions regarding uniformity of *Vitis vulpina.* The average in each case is of ten or more leaves from each vine.

TABLE 5. UNIFORMITY IN SIZE OF VEIN ISLETS IN EACH VINE

Number of vine	Minimum area of islets (square millimeters)	Average area (square millimeters)	Maximum area (square millimeters)
1	0.1843	0.2039	0.2175
2	0.3581	0.3684	0.3980
3	0.5001	0.5188	0.5288
4	0.2598	0.2700	0.2958

TABLE 5 (*concluded*)

Number of vine	Minimum area of islets (square millimeters)	Average area (square millimeters)	Maximum area (square millimeters)
31	0.3208	0.3337	0.3632
5	0.2819	0.2902	0.3129
6	0.1690	0.1807	0.1910
7	0.1221	0.1375	0.1634
8	0.2698	0.3250	0.3644
9	0.3170	0.3301	0.3453
10	0.2110	0.2291	0.2623
32	0.3500	0.4046	0.4400
33	0.2041	0.2312	0.2909
34	0.2961	0.3045	0.3178
35	0.3000	0.3140	0.3313
36	0.3810	0.3926	0.4061
37	0.4610	0.4743	0.4881
38	0.3097	0.3195	0.3309
39	0.2901	0.3077	0.3205
40	0.2593	0.2823	0.3001
41	0.1376	0.1541	0.1654
11	0.1534	0.1640	0.1751
12	0.3510	0.3946	0.4363
13	0.3489	0.3987	0.4511
15	0.3790	0.4657	0.5018
14	0.2598	0.2965	0.3321
16	0.3098	0.3280	0.3507
18	0.2106	0.2345	0.2511
17	0.4096	0.4845	0.5085
19	0.4597	0.5055	0.5410
21	0.3325	0.3690	0.3889
20	0.2217	0.2503	0.3053
22	0.3017	0.3311	0.3818
23	0.4885	0.5005	0.5171
24	0.3173	0.3553	0.3807
30	0.1337	0.1731	0.2311
Average	0.2961	0.3229	0.3496

The leaves with the smallest vein islets thus show sizes of islets 8.3 per cent below the average for the entire plant, while those with the largest show sizes of islets 8.3 per cent above the average. The degree of variation is distinctly less than that shown by the areas and weights of the leaves themselves, and is not greater than that commonly found in the numerical characters accepted in taxonomic work. This seems to indicate beyond reasonable doubt that the size of the vein islets is governed by some internal characteristic of the plant as a whole.

The way seems clear, therefore, to investigate the size of the vein islets as a possible indication of the age of the whole plant.

COMPARISON OF THE SIZE OF VEIN ISLETS IN PLANTS OF DIFFERENT AGES, IN VITIS VULPINA

For the purpose of determining whether the size of the vein islets of *Vitis vulpina* is dependent on the age of the whole plant, two methods of comparison were used: (1) a comparison of the size of vein islets in the leaves of these paired vines of different ages which were found growing under nearly identical external conditions; (2) a comparison of the size of the vein islets in the leaves borne by cuttings of vines of different ages which were grown under identical conditions in soil, in nutrient solutions, and in distilled water.

In addition to *Vitis vulpina*, *Vitis bicolor* Le Conte and a number of trees were investigated to a limited extent in regard to a possible relation between venation and age. The data on these latter are presented subsequently.

Size of vein islets of plants grown under field conditions

In the following tables (from table 6 to table 20) there are presented data on the vein islet measurements for each of ten leaves of each paired vine. In each table the average of the vein islet measurements for each of the paired vines is also given. The areas and weights of each leaf are shown. The age of the vine is indicated by the diameter of the main stem and by the number of annual rings.

TABLE 6. AREA OF VEIN ISLETS IN LEAVES OF VINES 1 AND 2

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 1, 7.6 centimeters in diameter, 28 annual rings					
1	13	16	151	2.5	0.2013
2	14	15	140	2.1	0.2112
3	15	19	207	3.6	0.2157
4	15	16	161	3.1	0.2050

TABLE 6 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 1, 7.6 centimeters in diameter, 28 annual rings (*concluded*)					
5	14	16	158	2.3	0.2171
6	16	16	178	2.8	0.1843
7	16	19	208	4.0	0.2028
8	15	17	175	3.2	0.2048
9	13	13	118	1.8	0.1941
10	13	15	130	3.2	0.2035
Vine 2, 2 centimeters in diameter, 6 annual rings					
1	13	15	138	2.3	0.3980
2	14	14	138	2.0	0.3716
3	13	15	137	2.2	0.3613
4	11	12	92	1.5	0.3581
5	13	14	124	2.0	0.3637
6	12	11	88	1.3	0.3696
7	12	14	118	1.9	0.3677
8	10	11	77	1.3	0.3691
9	10	11	74	1.2	0.3580
10	13	13	118	1.8	0.3670

Average area of islets for vine with 28 rings.................... 0.2040 square millimeter
Average area of islets for vine with 6 rings.................... 0.3684 square millimeter

TABLE 7. AREA OF VEIN ISLETS IN LEAVES OF VINES 3 AND 4

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 3, 0.7 centimeter in diameter, 3 annual rings					
1	15	17	190	2.3	0.5277
2	14	13	127	1.5	0.5151
3	17	18	210	2.2	0.5100
4	15	16	162	1.6	0.5180
5	15	16	162	1.7	0.5151
6	15	15	157	1.6	0.5288
7	14	16	152	1.8	0.5264
8	14	13	147	1.6	0.5001
9	15	17	173	2.0	0.5120
10	14	15	147	1.5	0.5010

TABLE 7 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 4, 7.6 centimeters in diameter, 25 annual rings					
1	12	15	116	1.9	0.2958
2	13	16	146	1.9	0.2598
3	14	14	133	1.7	0.2641
4	14	16	155	1.9	0.2698
5	12	14	119	1.3	0.2684
6	12	13	105	1.4	0.2710
7	10	12	84	1.0	0.2675
8	8	10	52	1.0	0.2720
9	9	10	63	1.0	0.2600
10	12	15	126	1.6	0.2640

Average area of islets for vine with 3 rings .... 0.5154 square millimeter
Average area of islets for vine with 25 rings .... 0.2692 square millimeter

TABLE 8. Area of Vein Islets in Leaves of Vines 5 and 6

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 5, 5.1 centimeters in diameter, 22 annual rings					
1	12	13	109	1.6	0.2813
2	12	15	126	1.6	0.2876
3	10	12	80	1.3	0.3129
4	8	13	72	1.8	0.2930
5	11	15	133	1.5	0.2818
6	13	15	140	1.8	0.2820
7	10	13	85	1.3	0.2912
8	13	11	97	1.4	0.2872
9	14	14	130	1.6	0.2953
10	14	16	152	1.8	0.2890
Vine 6, 20.4 centimeters in diameter, 56 annual rings					
1	11	11	85	0.8	0.1830
2	13	13	118	1.5	0.1910
3	14	14	132	1.4	0.1750
4	13	14	125	1.5	0.1810
5	10	11	74	0.7	0.1910
6	12	11	92	1.0	0.1756
7	11	12	92	0.9	0.1841

TABLE 8 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 6, 20.4 centimeters in diameter, 56 annual rings (*concluded*)					
8	12	11	85	1.0	0.1764
9	12	14	112	1.4	0.1690
10	13	13	110	1.2	0.1814

Average area of islets for vine with 22 rings	0.2901 square millimeter
Average area of islets for vine with 56 rings	0.1807 square millimeter

TABLE 9. AREA OF VEIN ISLETS IN LEAVES OF VINES 7 AND 8

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 7, 15.3 centimeters in diameter, 70 annual rings					
1	10	12	84	0.9	0.1335
2	12	14	117	0.9	0.1634
3	10	11	74	0.4	0.1238
4	9	10	60	0.3	0.1574
5	10	12	80	0.4	0.1221
6	10	11	77	0.5	0.1310
7	11	11	85	0.5	0.1360
8	10	11	74	0.4	0.1280
9	12	14	115	0.9	0.1412
10	13	14	120	0.8	0.1392
Vine 8, 3.2 centimeters in diameter, 15 annual rings					
1	10	10	70	0.6	0.2866
2	10	11	74	0.6	0.2698
3	11	12	89	0.7	0.3644
4	13	12	109	0.8	0.3525
5	9	9	57	0.4	0.3166
6	11	11	87	0.6	0.3270
7	11	12	90	0.6	0.3460
8	11	11	80	0.7	0.3272
9	8	9	46	0.4	0.3360
10	9	9	57	0.5	0.3241

Average area of islets for vine with 70 rings	0.1376 square millimeter
Average area of islets for vine with 15 rings	0.3250 square millimeter

TABLE 10. AREA OF VEIN ISLETS IN LEAVES OF VINES 9 AND 10

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 9, 3.3 centimeters in diameter, 14 annual rings					
1	14	15	140	1.5	0.3390
2	14	15	147	1.6	0.3453
3	13	13	112	1.2	0.3292
4	14	14	140	1.4	0.3390
5	11	11	85	0.8	0.3355
6	12	11	90	0.8	0.3240
7	10	12	84	0.7	0.3172
8	15	18	182	2.0	0.3361
9	14	12	118	1.2	0.3170
10	11	13	100	0.8	0.3211
Vine 10, 7.6 centimeters in diameter, 30 annual rings					
1	10	11	77	0.6	0.2141
2	10	10	70	0.7	0.2623
3	14	12	112	1.7	0.2460
4	15	17	170	2.4	0.2350
5	15	15	154	1.8	0.2228
6	11	12	87	0.7	0.2241
7	14	15	147	1.8	0.2342
8	15	16	162	2.0	0.2110
9	15	15	158	1.9	0.2151
10	11	13	90	0.9	0.2262

Average area of islets for vine with 14 rings.................. 0.3303 square millimeter
Average area of islets for vine with 30 rings.................. 0.2291 square millimeter

TABLE 11. AREA OF VEIN ISLETS IN LEAVES OF VINES 11 AND 12

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 11, 1.3 centimeters in diameter, 6 annual rings (only nine leaves picked)					
1	14	12	110	1.3	0.3617
2	17	15	170	2.0	0.4246
3	17	14	155	1.7	0.4363
4	17	16	180	2.1	0.3510
5	15	13	130	1.5	0.3571

TABLE 11 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 11, 1.3 centimeters in diameter, 6 annual rings (only nine leaves picked) (*concluded*)					
6	15	13	140	1.6	0.4006
7	14	11	98	1.0	0.4213
8	17	16	190	2.2	0.4313
9	15	12	126	1.4	0.3679
Vine 12, 15.3 centimeters in diameter, 57 annual rings					
1	9	10	60	0.8	0.1543
2	9	9	54	0.7	0.1678
3	10	10	66	0.7	0.1751
4	10	10	73	0.9	0.1642
5	9	8	48	0.6	0.1657
6	9	10	60	0.7	0.1635
7	8	7	40	0.5	0.1706
8	10	9	63	0.7	0.1613
9	8	8	41	0.4	0.1534
10	8	7	40	0.5	0.1642

Average area of islets for vine with 6 rings.................. 0.3946 square millimeter
Average area of islets for vine with 57 rings.................. 0.1640 square millimeter

TABLE 12. AREA OF VEIN ISLETS IN LEAVES OF VINES 13 AND 14

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 13, 1.3 centimeters in diameter, 6 annual rings (only five leaves picked)					
1	13	12	109	1.2	0.3597
2	15	10	108	1.1	0.4213
3	12	10	84	0.9	0.3489
4	10	9	65	0.7	0.4106
5	8	5	30	0.4	0.4511
Vine 14, 5.1 centimeters in diameter, 16 annual rings					
1	16	16	180	2.1	0.3209
2	10	10	70	0.9	0.2871
3	13	12	110	1.2	0.2696
4	13	13	112	1.2	0.3213

TABLE 12 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 14, 5.1 centimeters in diameter, 16 annual rings (*concluded*)					
5	12	12	100	1.0	0.2871
6	14	15	150	1.7	0.3107
7	18	16	210	2.3	0.2879
8	12	12	106	1.1	0.3321
9	14	15	145	1.6	0.2897
10	14	14	135	1.5	0.2598

Average area of islets for vine with 6 rings .... 0.3983 square millimeter
Average area of islets for vine with 16 rings .... 0.2966 square millimeter

TABLE 13. Area of Vein Islets in Leaves of Vines 15 and 16

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 15, 0.8 centimeter in diameter, 5 annual rings (only five leaves picked)					
1	10	9	60	0.6	0.3790
2	9	8	54	0.6	0.5013
3	10	10	73	0.8	0.4678
4	11	9	70	0.8	0.5018
5	12	10	84	0.9	0.4787
Vine 16, 5.1 centimeters in diameter, 14 annual rings					
1	14	13	135	1.5	0.3312
2	14	15	145	1.6	0.3176
3	13	11	105	1.2	0.3098
4	14	16	150	1.7	0.3421
5	15	14	150	1.6	0.3507
6	12	13	110	1.2	0.3312
7	14	14	135	1.5	0.3212
8	13	12	107	1.1	0.3106
9	12	12	106	1.1	0.3361
10	13	12	110	1.2	0.3301

Average area of islets for vine with 5 rings .... 0.4657 square millimeter
Average area of islets for vine with 14 rings .... 0.3281 square millimeter

TABLE 14. AREA OF VEIN ISLETS IN LEAVES OF VINES 17 AND 18

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 17, 1.3 centimeters in diameter, 5 annual rings (only eight leaves picked)					
1	12	11	90	0.9	0.4863
2	12	12	100	1.1	0.5017
3	12	10	78	0.9	0.4096
4	12	13	104	1.1	0.5023
5	12	10	80	0.9	0.5085
6	11	12	85	0.9	0.4893
7	10	10	75	0.8	0.4971
8	11	10	75	0.9	0.4813
Vine 18, 10.1 centimeters in diameter, 35 annual rings					
1	14	15	150	1.7	0.2417
2	14	13	130	1.4	0.2378
3	15	14	135	1.5	0.2363
4	14	14	125	1.4	0.2419
5	13	13	115	1.3	0.2263
6	13	12	110	1.2	0.2511
7	11	14	110	1.3	0.2387
8	12	11	90	1.0	0.2267
9	11	13	100	1.1	0.2239
10	10	13	90	1.0	0.2106

Average area of islets for vine with 5 rings.................. 0.4845 square millimeter
Average area of islets for vine with 35 rings.................. 0.2335 square millimeter

TABLE 15. AREA OF VEIN ISLETS IN LEAVES OF VINES 19 AND 20

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 19, 1.5 centimeters in diameter, 5 annual rings					
1	15	13	110	1.2	0.5410
2	13	12	105	1.1	0.5363
3	14	13	140	1.6	0.4597
4	13	12	115	1.2	0.4817
5	15	13	135	1.6	0.4861
6	16	15	170	1.9	0.4877
7	14	13	125	1.4	0.5437
8	13	13	120	1.4	0.5019
9	12	12	105	1.3	0.5115
10	11	12	100	1.2	0.5061

TABLE 15 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 20, 10.1 centimeters in diameter, 25 annual rings					
1	8	7	38	0.5	0.2509
2	8	8	45	0.6	0.2263
3	9	7	50	0.6	0.2217
4	9	11	65	0.7	0.3053
5	11	10	75	0.8	0.2506
6	8	9	65	0.8	0.2618
7	7	7	45	0.6	0.2791
8	11	12	85	1.0	0.2507
9	11	10	85	0.9	0.2273
10	10	10	75	0.9	0.2291

Average area of islets for vine with 5 rings	0.5056 square millimeter
Average area of islets for vine with 25 rings	0.2503 square millimeter

TABLE 16. AREA OF VEIN ISLETS IN LEAVES OF VINES 21 AND 22

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 21, 2.5 centimeters in diameter, 11 annual rings					
1	13	14	125	1.4	0.3191
2	11	12	90	1.0	0.3889
3	13	14	120	1.3	0.3325
4	12	12	140	1.6	0.3835
5	12	13	110	1.2	0.3896
6	12	14	120	1.4	0.3764
7	10	12	95	1.1	0.3761
8	13	13	100	1.2	0.3673
9	10	11	80	0.9	0.3809
10	10	11	85	0.9	0.3761
Vine 22, 5.1 centimeters in diameter, 17 annual rings					
1	17	17	202	2.2	0.3087
2	17	18	216	2.4	0.3818
3	15	15	158	1.8	0.3506
4	14	14	135	1.5	0.3017
5	15	15	160	1.8	0.3063
6	15	14	150	1.7	0.3219

TABLE 16 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 22, 5.1 centimeters in diameter, 17 annual rings (*concluded*)					
7	15	14	145	1.6	0.3367
8	15	15	160	1.8	0.3503
9	15	15	165	1.9	0.3307
10	14	14	130	1.5	0.3218

Average area of islets for vine with 11 rings .... 0.3690 square millimeter
Average area of islets for vine with 17 rings .... 0.3310 square millimeter

TABLE 17. AREA OF VEIN ISLETS IN LEAVES OF VINES 23 AND 24

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 23, 0.8 centimeter in diameter, 5 annual rings (only nine leaves picked)					
1	11	11	80	0.9	0.4893
2	12	10	80	0.9	0.4972
3	10	12	85	0.9	0.4885
4	11	11	85	0.9	0.5006
5	10	12	90	1.0	0.5097
6	10	11	70	0.8	0.4998
7	9	9	60	0.7	0.5013
8	9	7	50	0.6	0.5171
9	10	11	75	0.8	0.4909
Vine 24, 2.5 centimeters in diameter, 10 annual rings					
1	12	11	100	1.1	0.3709
2	10	11	75	0.9	0.3761
3	12	13	115	1.3	0.3781
4	11	12	101	1.1	0.3407
5	10	11	70	0.8	0.3173
6	10	11	75	0.9	0.3164
7	10	11	80	0.9	0.3187
8	11	13	95	1.0	0.3861
9	11	12	95	1.0	0.3275
10	11	12	100	1.1	0.3209

Average area of islets for vine with 5 rings .... 0.4994 square millimeter
Average area of islets for vine with 10 rings .... 0.3453 square millimeter

TABLE 18. AREA OF VEIN ISLETS IN LEAVES OF VINES 25 AND 26

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 25, 3 centimeters in diameter, 11 annual rings (only five leaves photographed)					
1	13	14	125	1.4	0.3761
2	14	13	130	1.5	0.3506
3	14	15	145	1.6	0.3391
4	13	15	135	1.5	0.3476
5	12	13	100	1.1	0.3736
Vine 26, 12.7 centimeters in diameter, 35 annual rings (only five leaves photographed)					
1	12	11	90	1.0	0.2216
2	13	13	100	1.1	0.2035
3	14	15	150	1.7	0.2107
4	11	13	100	1.1	0.2316
5	12	13	110	1.2	0.2217

Average area of islets for vine with 11 rings .... 0.3574 square millimeter
Average area of islets for vine with 35 rings .... 0.2178 square millimeter

TABLE 19. AREA OF VEIN ISLETS IN LEAVES OF VINES 27 AND 28

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 27, 5.1 centimeters in diameter, 17 annual rings (only five leaves photographed)					
1	15	15	150	1.7	0.3281
2	13	12	110	1.3	0.3011
3	16	15	165	1.8	0.3132
4	13	13	110	1.3	0.3313
5	13	13	115	1.2	0.3063
Vine 28, 7.6 centimeters in diameter, 25 annual rings (only five leaves photographed)					
1	11	11	85	0.9	0.2261
2	12	11	90	1.0	0.2306
3	14	13	125	1.4	0.2413
4	13	10	100	1.1	0.2371
5	15	13	125	1.4	0.2283

Average area of islets for vine with 17 rings .... 0.3160 square millimeter
Average area of islets for vine with 25 rings .... 0.2327 square millimeter

TABLE 20. AREA OF VEIN ISLETS IN LEAVES OF VINES 29 AND 30

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 29, 2.5 centimeters in diameter, 10 annual rings (only five leaves photographed)					
1	11	12	90	1.1	0.3613
2	14	15	145	1.6	0.3725
3	13	13	115	1.3	0.3617
4	15	14	135	1.5	0.3867
5	16	15	155	1.7	0.3975
Vine 30, 12.7 centimeters in diameter, 50 annual rings					
1	8	7	42	0.5	0.2013
2	8	8	50	0.6	0.1809
3	8	6	40	0.5	0.2311
4	8	7	45	0.6	0.2263
5	10	10	70	0.8	0.1491
6	9	9	60	0.7	0.1347
7	9	10	64	0.7	0.1509
8	9	9	62	0.7	0.1814
9	10	10	70	0.8	0.1337
10	10	9	60	0.7	0.1416

Average area of islets for vine with 10 rings	0.3759 square millimeter
Average area of islets for vine with 50 rings	0.1731 square millimeter

TABLE 21. SUMMARY OF RELATION FOUND BETWEEN AGE OF VINE OF VITIS VULPINA AND AREA OF VEIN ISLETS IN THE LEAVES

Number of vine	Number of annual rings	Area of islets (square millimeters)	Number of vine	Number of annual rings	Area of islets (square millimeters)
3	3	0.5154	14	16	0.2966
19	5	0.5056	27	17	0.3160
23	5	0.4994	22	17	0.3310
17	5	0.4845	5	22	0.2901
15	5	0.4657	4	25	0.2692
13	6	0.3983	20	25	0.2503
11	6	0.3946	28	25	0.2327
2	6	0.3684	1	28	0.2040
29	10	0.3759	10	30	0.2291
24	10	0.3453	18	35	0.2335
21	11	0.3690	26	35	0.2178
25	11	0.3574	30	50	0.1731
9	14	0.3303	6	56	0.1807
16	14	0.3281	12	57	0.1640
8	15	0.3250	7	70	0.1376

By averaging the size of the vein islets in vines of the same age, a curve can be plotted which shows very strikingly the decrease in the size of the islets with increasing age. Such a curve is shown in figure 55.

When the plants in the above summary are divided into groups the change in venation is even more apparent, as shown in table 22.

In figure 56 are shown photographs of the venation characteristic of each of these groups.

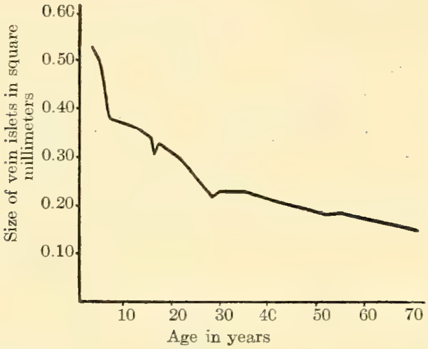

FIG. 55.— *Curve showing decrease with age in size of vein islets of leaves of Vitis vulpina*

TABLE 22. AVERAGE AREA OF VEIN ISLETS IN LEAVES OF VINES GROUPED ACCORDING TO AGE

Number of rings	3 to 5	6 to 11	14 to 25	28 to 35	50 to 70
Area of vein islets (square millimeters)	0.4941	0.3727	0.2969	0.2211	0.1638

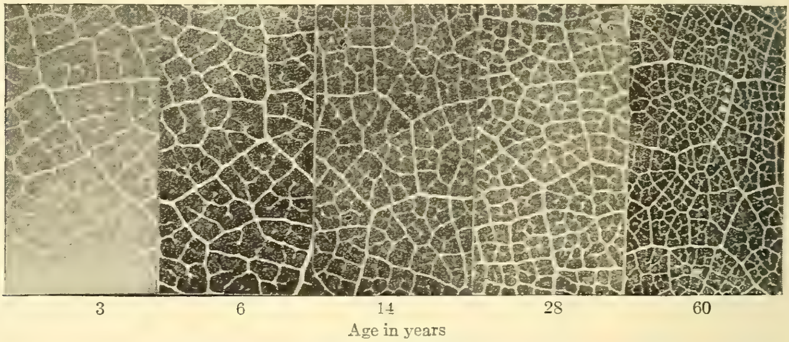

FIG. 56.—*Photographs of vein islets of leaves of Vitis vulpina vines of the ages of 3, 6, 14, 28, and 60 years, grown under identical conditions*

Size of islets in leaves of cuttings grown under identical conditions

In order to test further the constancy of the different sizes of vein islets found, cuttings were made, in three different summers, from the various vines studied. These cuttings were grown in water and in soil cultures, and the leaves that developed were measured and studied. The cuttings were classified into groups according to the age of the vine from which they were taken, and were labeled accordingly but grown together under identical conditions.

Of the first series of cuttings grown in tap water, seventeen proved successful. They were grown in a greenhouse at Ithaca. Since it was necessary for the writer to go to Cincinnati at the close of the summer, the measurements of the vein islets were made before the leaves had attained maturity. The results of these measurements are given in table 23. The number of rings as given in the table indicates the age of the vine from which the cuttings were made; the size given for the vein islets is the average for all the leaves of one cutting.

TABLE 23. Area of Vein Islets in Leaves from Cuttings Grown in Tap Water

	Number of rings			
	4 to 5	7 to 11	14 to 16	25 to 50
	(Square millimeters)	(Square millimeters)	(Square millimeters)	(Square millimeters)
	0.24	0.20	0.16	0.14
	0.26	0.21	0.22	0.15
	0.29	0.22	0.17	0.15
	0.30	0.23	0.18	0.15
	....	0.32	....	....
Average.....	0.27	0.24	0.18	0.15

The vein islets are noticeably smaller than those in leaves grown under natural conditions, and this is due to the fact that the leaves were not fully expanded. The smaller the leaf, the smaller are the islets until the maturity of the leaf is reached. These differences in size of vein islets of leaves of old and of young vines are obvious at the time when the leaf buds first open, and are maintained throughout the later stages of leaf growth, as is shown by tables 24 and 25, pages 314 and 315.

Comparison of the venation of immature leaves

In order to determine whether or not the difference in size of vein islets is present from the beginning of the development of the leaf, the venation of a series of leaves from the tips of shoots backward was examined. One examination was made by the photographic method, with the following results:

TABLE 24. Area of Vein Islets in a Series of Immature Leaves Taken in Order from the Tips of the Shoots Backward, from Vitis vulpina Twenty-five and Ten Years of Age

Number of leaf	Length (centimeters)	Breadth (centimeters)	Weight (grams)	Area of vein islets (square millimeters)
		Vine twenty-five years old		
1	3	3	0.15	0.010
2	5	4	0.30	0.015
3	7	6	0.50	0.022
4	8	9	0.60	0.058
5	11	10	0.95	0.090
6	11	12	1.10	0.120
7	14	17	2.10	0.150
8	13	14	1.90	0.190
		Vine ten years old		
1	3	3	0.16	0.015
2	6	5	0.29	0.020
3	8	6	0.51	0.027
4	9	8	0.63	0.065
5	10	11	1.00	0.098
6	11	12	1.20	0.150
7	14	17	2.20	0.200
8	13	14	2.00	0.260

That this was an entirely typical condition has been shown by many field counts of veinlet intersections by a method explained later (page 317). The sizes of the vein islets in the immature leaves on the cuttings as given in table 23 offer evidence to the same effect.

If the midrib be called the primary vein, its chief branches secondary veins, the main branches of the secondary veins tertiary, and so on, the final most minute veinlets may be given a definite rank. A comparison of the rank of the veinlets in leaves borne by cuttings of *Vitis vulpina* of different ages shows the following results:

TABLE 25. RANK OF VEINLETS IN IMMATURE LEAVES BORNE BY CUTTINGS OF VITIS VULPINA OF DIFFERENT AGES

Position of leaf	Enclosing veinlets	Penetrating veinlets
Vine twelve years old		
First leaf on cuttings	5th rank	6th rank
Second leaf	6th rank	7th rank
Third leaf	6th rank	7th rank
Fourth leaf	6th rank	7th rank
Fifth leaf	7th rank	8th rank
Sixth leaf	7th rank	8th rank
Seventh leaf	7th rank	8th rank
Vine twenty-six years old		
First leaf	6th rank	7th rank
Second leaf	7th rank	8th rank
Third leaf	6th rank	7th rank
Fourth leaf	7th rank	7th rank
Fifth leaf	7th rank	8th rank
Sixth leaf	8th rank	9th rank
Seventh leaf	8th rank	9th rank

The important fact is evident from table 25 that in the leaf from the older vine the vein islets have been divided by the development of an additional rank of veinlets, and each division has been partly penetrated by veinlets of still higher rank. The fact that size relationship of the vein islets holds for the immature leaves of old and young vines as definitely as it does for the mature ones, which have been subjected to the various external factors of environment, is further evidence of importance supporting the claim that the difference in size of the vein islets is due to a constitutional rather than to an environmental cause.

In May, 1910, another set of cuttings was started, this time in prepared soil. These cuttings were obtained and started for the writer by an assistant, and the grouping was somewhat different from that used in the first series. All the cuttings were grown in the same box of prepared soil and all environmental conditions were identical for each cutting. The cuttings produced mature leaves in time for study during the summer. The average size of the vein islets in the leaves of each of these fifty-four cuttings is shown in table 26, the number of rings indicating the age in years of the vine from which the cuttings were made. The cuttings themselves varied in diameter from 1.3 to 2.5 centimeters, both small and large cuttings being taken from the older vines. The size of the vein islets had no relation to the size of the cuttings.

TABLE 26. AREA OF VEIN ISLETS IN LEAVES FROM CUTTINGS GROWN IN SOIL

	Number of rings		
	5 to 15	16 to 25	26 to 40
	(Square millimeters)	(Square millimeters)	(Square millimeters)
	0.52	0.37	0.32
	0.50	0.37	0.33
	0.49	0.35	0.28
	0.41	0.34	0.30
	0.40	0.35	0.36
	0.36	0.35	0.38
	0.34	0.30	0.38
	0.36	0.37	0.34
	0.44	0.34	0.40
	0.40	0.40	0.32
	0.50	....	0.35
	0.49	....	0.31
	0.49	....	0.23
	0.48	....	0.26
	0.48	....	0.26
	0.47	....	0.33
	0.42	....	0.33
	0.40	....	0.23
	0.42	....	0.23
	0.36	....	0.20
	....	....	0.23
	....	....	0.24
	....	....	0.23
	....	....	0.23
Average........	0.44	0.35	0.29

The relation between the size of vein islets in these cuttings and the age of the original vines is graphically shown in curve A of figure 57.

It is possible that many of the cuttings came from vines on the boundary lines of the age groups, instead of being evenly distributed throughout the whole range of each group; but, in spite of this, the inherent effect of the age of the vine is plainly seen.

METHOD FOR DETERMINING APPROXIMATE AGE BY MEASUREMENT OF VEINAGE

In addition to the foregoing investigation, hundreds of field measurements of the relation between the size of islets and the age of the vine were made, in five different summers, about Cincinnati and Ithaca. The method used was simple, and well adapted to the practical nurseryman and fruit grower. A paper clip (called the cub tiger clip) was procured and a distance of two centimeters marked along the jaws. A piece of the leaf to be examined was torn out from between two large secondary veins, held by the clip toward the light, and examined with a pocket lens. The number of veinlets intersecting the edge of the jaw in a distance of two centimeters was counted in several places on the leaf and the average was taken. The result of these observations showed that such an average gives a rough approximation of the age of the vine on which leaves have grown, as indicated in table 27:

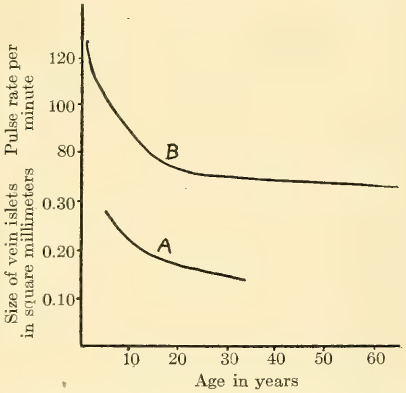

FIG. 57.—*Curves showing (A) senile decrease in size of vein islets in leaves borne by cuttings of Vitis vulpina, and (B) senile decrease in pulse rate in human beings*

TABLE 27. FIELD METHOD OF DETERMINING THE APPROXIMATE AGE OF VITIS VULPINA VINES FROM THE SIZE OF THE VEIN ISLETS IN THE LEAVES

Number of veinlets intersecting a straight line in two centimeters	Corresponding age of vine
30 to 35	5 years or less
35 to 45	5 to 15 years
45 to 50	15 to 35 years
50 to 75	35 years and upward

While this method is, of course, not so free from error as the photographic method — since only a fraction of the actual number of islets are measured and a lineal instead of an area calculation is employed — still it is a practical method for general use in field, nursery, and orchard, such as was originally sought.

In about one per cent of the several hundred field determinations made and checked by ring counts, serious discrepancies were found. It is possible that these exceptional vines were examples of such extreme variations as are sometimes found in other specific morphological characters. A number of other species of perennial plants were examined, and it was found that, while the size of vein islets always diminished with age, the actual size of the islets at any age was a specific character relatively constant in each species. Such individual abnormal plants as those mentioned above resemble other species in the character of their venation.

COMPARISON OF SIZE OF VEIN ISLETS IN PLANTS OF DIFFERENT AGES, IN VITIS BICOLOR AND CERTAIN OTHER PLANTS

The results of a preliminary investigation of the relation between age and venation in some woody perennials other than *Vitis vulpina* are given in the following pages.

Size of vein islets in Vitis bicolor Le Conte

The vines used for these measurements were not transversely cut, and the only guide that could be obtained to their relative ages was the diameter of the stems. They were all growing in the same small area, under apparently identical conditions.

TABLE 28. Area of Vein Islets in Leaves of Vitis bicolor, Vines 1 and 2

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
			Vine 1, 25.5 centimeters in diameter		
1	17	18	200	2.0	0.2276
2	18	17	210	2.7	0.2298
3	16	15	170	2.6	0.2909
4	18	18	220	3.5	0.2391

TABLE 28 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 1, 25.5 centimeters in diameter (*concluded*)					
5	18	17	220	2.8	0.2256
6	14	15	147	2.0	0.2127
7	17	18	210	2.8	0.2041
8	17	20	230	3.4	0.2113
9	14	17	170	2.6	0.2418
10	19	19	260	2.6	0.2291
Vine 2, 3.8 centimeters in diameter					
1	13	15	140	2.1	0.3810
2	12	13	105	1.5	0.4061
3	13	15	135	2.2	0.3818
4	15	15	155	2.2	0.3819
5	13	14	125	2.1	0.4021
6	10	13	90	1.1	0.3971
7	14	15	145	2.1	0.3818
8	12	12	100	1.2	0.3981
9	11	12	85	1.0	0.4001
10	12	13	105	1.5	0.3961

Average area of islets for vine 25.5 centimeters in diameter...... 0.2312 square millimeter
Average area of islets for vine 3.8 centimeters in diameter....... 0.3926 square millimeter

TABLE 29. AREA OF VEIN ISLETS IN LEAVES OF VITIS BICOLOR, VINES 3 AND 4

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 3, 15.3 centimeters in diameter					
1	12	18	160	3.0	0.3066
2	13	17	165	3.0	0.3170
3	14	15	150	2.9	0.3001
4	13	17	165	3.0	0.3101
5	13	14	115	2.6	0.2909
6	13	16	145	2.5	0.2961
7	12	16	120	2.7	0.3106
8	14	15	145	3.1	0.2989
9	13	16	150	2.9	0.3179
10	13	16	145	2.8	0.3091

TABLE 29 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 4, 2.5 centimeters in diameter					
1	14	16	160	2.2	0.4687
2	13	16	155	2.5	0.4865
3	13	16	145	2.1	0.4671
4	14	16	160	2.4	0.4881
5	14	12	125	2.0	0.4652
6	15	17	180	2.5	0.4721
7	15	17	185	2.1	0.4863
8	14	17	170	2.6	0.4769
9	13	17	160	2.2	0.4610
10	13	14	115	1.9	0.4713

Average area of islets for vine 15.3 centimeters in diameter...... 0.3057 square millimeter
Average area of islets for vine 2.5 centimeters in diameter........ 0.4743 square millimeter

TABLE 30. AREA OF VEIN ISLETS IN LEAVES OF VITIS BICOLOR, VINES 5 AND 6

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 5, 20.4 centimeters in diameter					
1	13	16	155	2.4	0.3313
2	14	14	140	1.7	0.3000
3	14	16	160	2.5	0.3001
4	13	15	135	2.2	0.3139
5	12	15	120	2.0	0.3294
6	14	15	145	2.0	0.3012
7	13	16	140	2.4	0.3116
8	14	13	135	1.8	0.3285
9	11	15	120	1.8	0.3017
10	12	14	120	1.4	0.3123
Vine 6, 4.5 centimeters in diameter					
1	11	14	110	1.3	0.3713
2	10	14	105	1.2	0.2867
3	10	14	100	0.8	0.3873
4	10	12	90	1.2	0.4013
5	12	13	110	1.7	0.3764
6	12	14	115	1.7	0.3971
7	13	14	130	1.9	0.3865
8	13	15	135	2.0	0.3871

TABLE 30 (*concluded*)

Number of leaf	Length (centimeters)	Breadth (centimeters)	Area (square centimeters)	Weight (grams)	Area of islets (square millimeters)
Vine 6, 4.5 centimeters in diameter (*concluded*)					
9	11	16	115	1.8	0.3763
10	13	14	135	0.8	0.3705

Average area of islets for vine 20.4 centimeters in diameter	0.3130 square millimeter
Average area of islets for vine 4.5 centimeters in diameter	0.3740 square millimeter

TABLE 31. SUMMARY OF DATA FOR VITIS BICOLOR LE CONTE

Diameter of vine (in centimeters)	2.5	3.8	4.5	15.3	20.4	25.5
Size of islets (in square millimeters)	0.4743	0.3926	0.3740	0.3057	0.3130	0.2312

This species is characterized by larger vein islets than those of *Vitis vulpina*, but exhibits the same age-venation relationship.

Size of vein islets in Tecoma radicans L.

The vines of *Tecoma radicans* examined were growing in a private yard. Those that were 20.4 centimeters in diameter had been planted, and the smaller ones had come up from their seeds. The size of islets given is the average for ten leaves of each plant. Here again the vines were not cut and the age must be inferred from the diameter of the stem.

TABLE 32. AREA OF VEIN ISLETS IN TECOMA RADICANS

	Diameter of stem		
	1.3 centimeters	3.8 centimeters	20.4 centimeters
	Square millimeters	Square millimeters	Square millimeters
	1.5	0.95	0.62
	1.4	0.97	0.63
	1.5	0.94	0.68
	1.6	0.98	0.70
	....	0.96	0.67
Average	1.5	0.96	0.66

In this case, as in the case of *Vitis vulpina* and *V. bicolor*, the more rapid decrease in size of vein islets in the early years is to be particularly noted. The significance of this will be discussed later.

Another interesting observation was made with these specimens of *Tecoma radicans*. Among the seedlings that sprouted around the parent vines, root shoots from the old vines were found growing. The connection of these root shoots was established by examination. Although seedlings and root shoots seemed equally vigorous and could not be distinguished from each other at first sight, a venation count successfully separated them, since the root shoots all showed in their leaves the size of vein islets characteristic of the parent plant, while those of the seedlings were much larger. This fact is of considerable importance in its bearing on the cause of these differences, as will be indicated later.

Size of vein islets in several kinds of trees

The following data, as is evident, represent nothing but a comparison of a few trees of different ages that were growing under similar external conditions, and the results are interesting merely as a corroboration of the effect of age in decreasing the size of vein islets in perennials:

TABLE 33. RELATION OF SIZE OF VEIN ISLETS TO AGE IN SALIX NIGRA MARSH

Diameter of trunk (in centimeters)	2.5	15.3	38.1
Area of islets (in square millimeters)	0.55	0.4	0.3

The largest tree was probably the parent of the others, as they grew in an isolated place.

TABLE 34. RELATION OF SIZE OF VEIN ISLETS TO AGE IN CASTANEA DENTATA BORKH.

Diameter of trunk (in centimeters)	5.1	12.7	25.5	61.2	91.2
Area of islets (in square millimeters)	1.0	0.7	0.5	0.4*	0.3

* The tree 61.2 centimeters in diameter had been cut down, and the leaves examined were very large ones borne on luxuriant water sprouts.

TABLE 35. RELATION OF SIZE OF VEIN ISLETS TO AGE IN QUERCUS ALBA L.

Diameter of trunk (in centimeters)	0.7	40.8	38.0
Area of islets (in square millimeters)	1.5	0.3*	0.3

* The leaves examined were much larger than normal size, being borne on luxuriant water sprouts.

TABLE 36. Relation of Size of Vein Islets to Age in Tilia americana L.

Diameter of trunk (in centimeters)	7.6	35.7
Area of leaf (in square centimeters)	30.0	1,560.0
Area of islets (in square millimeters)	0.04	0.02

In the latter case the leaves were borne by water sprouts growing from the stump and they were fifty times the size of the leaves on the small tree; yet the size of the vein islets corresponded to the age of the plant.

TABLE 37. Relation of Size of Vein Islets to Age in Ulmus americana L.

Diameter of trunk (in centimeters)	30.6	35.7*
Area of islets (in square millimeters)	0.8	0.6

The use of the field method is shown in the following figures obtained from the same species:

Diameter of trunk (in centimeters)	2.5	5.1	25.5	61.2
Number of intersections in 2 centimeters	38	48	54	94

*Stump with water-sprouts.

TABLE 38. Relation of Size of Vein Islets to Age in Carya alba Koch

Diameter of trunk (in centimeters)	22.8	45.6
Number of intersections in 2 centimeters	40	60–62

TABLE 39. Relation of Size of Vein Islets to Age in Carya ovata Koch

Diameter of trunk (in centimeters)	2.5	3.8	20.4	45.6
Number of intersections in 2 centimeters	50	56	72	88

TABLE 40. Relation of Size of Vein Islets to Age in Acer saccharium L.

Diameter of trunk (in centimeters)	1.3	2.5	30.6	38
Number of intersections in 2 centimeters	42	52	80	88–94

TABLE 41. RELATION OF SIZE OF VEIN ISLETS TO AGE IN ACER SACCHARUM MARSH

Diameter of trunk (in centimeters)	2.5	45.9	61.2
Number of intersections in 2 centimeters	50	74	90

TABLE 42. RELATION OF SIZE OF VEIN ISLETS TO AGE IN QUERCUS VELUTINA LAM.

Diameter of trunk (in centimeters)	2	3.8	76
Number of intersections in 2 centimeters	50	58	84

TABLE 43. RELATION OF SIZE OF VEIN ISLETS TO AGE IN PLATANUS OCCIDENTALIS L.

Diameter of trunk (in centimeters)	5.1	10.2	20.6	45.6
Number of intersections in 2 centimeters	50	64	68	74

TABLE 44. RELATION OF SIZE OF VEIN ISLETS TO AGE IN FRAXINUS AMERICANA L.

Diameter of trunk (in centimeters)	1.3	30.6
Number of intersections in 2 centimeters	40	80

Size of vein islets in leaves of several varieties of fruits propagated by cuttings

If age affects meristematic tissue in seed-producing plants, the cells of the scions used in propagating certain varieties of fruits must be affected. Unless the process of grafting and growth on seedling stocks restores the youthful condition of the protoplasm of the scion, its leaves should show a size of vein islets typical of the time that has elapsed since the variety originated from seed, not of the time since the graft was made. The application of this venation test in order to determine the physiological condition of present cultivated varieties is most important, but lies beyond the limits of the present paper. However, a few preliminary tests were made of the relative sizes of vein islets in several varieties of cultivated grapes that have been in existence for different lengths of time. Through the kindness of Dr. U. P. Hedrick, of the New York State Agricultural Experiment Station at Geneva, the pedigreed vines growing there were placed at the writer's disposal, and table 45 is based on them.[3] The vines of Concord, Worden, Catawba, and Diana were of equal size, and all the varieties were growing under apparently identical conditions of environment.

TABLE 45. SIZE OF VEIN ISLETS IN LEAVES OF SIX VARIETIES OF CULTIVATED GRAPES

Variety	Years since origin	Age of individual plant examined	Number of intersecting veinlets in 2 centimeters
Concord	70	14	66
Worden (Concord seedling)	50	14	58
Concord seedling	7	7	35
Catawba	114	14	82
Diana (Catawba seedling)	63	14	74
Diana seedling	5	5	38

The size of the vein islet in these determinations is evidently related to the time that has elapsed since the last seed propagation. Although in the case of four of these varieties many generations of cutting propagation had intervened, in each of which meristematic tissue had been removed from contact with old specialized tissues, nevertheless the decrease with age in size of the vein islets had progressed in much the same way as if contact with the first individual of the variety had been maintained.

Further applications of the venation test to cultivated plants will be discussed subsequently in this paper. (See page 351.)

CONCLUSIONS REGARDING THE SIZE OF VEIN ISLETS AS EVIDENCE OF THE OCCURRENCE OF TRUE SENILITY IN PERENNIAL PLANTS

The results of this study of the leaf venation of the woody perennials mentioned have demonstrated that the size of the vein islets constantly decreases as the plant grows older. The only physical connection between the leaves of this year and those of past years is the meristematic tissue from which all are developed in their time. If this change in the size of the vein islets is the effect of progressing senility, then the constant changes must be occurring in the meristematic tissue of the plant, and it follows that this tissue must no longer be considered immune from senile degeneration and from death.

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[3] The writer desires to thank M. J. Dorsey and R. D. Anthony for their assistance in locating cultivated plants used, and for the data regarding the ages of the varieties and individuals.

There remains to be examined the possibility that the change in size of the vein islets may be due to some cause other than the progressive senile degeneration of the meristematic tissue. The possible causes may be grouped into two divisions: first, changes in the old specialized tissues of perennial plants; second, differences in external conditions resulting from increasing size and subsequent difference in position of leaves.

Is it possible that some progressive change occurring in the early specialized tissues of the root or the stem produces the decrease in size of the vein islets, and therefore no senile degeneration of meristematic tissue need be considered? Any such progressive change either must interfere with the transfer of materials to and from the meristem and the developing leaf, or must act through the production of toxic products that poison the meristem and the leaf.

If the decrease in size of the vein islets is caused by a progressive decrease in the efficiency of the root or the stem as absorbing and conducting organs, then not only the venation, but the size and development of the leaf as well, should be affected. The leaves and the shoots of *Vitis vulpina*, however, show absolutely no signs of impaired vigor of growth. In fact, the leaves and the shoots of very young vines often appear smaller and less luxuriant than those of older vines, due, no doubt, to differences of nutrition received. Furthermore, data given show no relationship between the sizes of the leaves and the sizes of vein islets. The water shoots from the stumps of cut trees and the root shoots of Tecoma, mentioned above, must have had much more than the normal supply of nutritive materials, but this did not markedly change the size of the vein islets. It was found also that leaves borne by cuttings grown under identical conditions showed the same differences in venation as were present on the original vines in their natural habitat.

It should not be overlooked in this connection that the leaves are not the only new structures being constantly produced in perennials. The increase in number of root hairs keeps pace with the number of leaves, and the increasing circumference of the newly formed zones of xylem and phloëm tends to keep the supply up to the demand. In most perennials conduction occurs largely through tissues that have been specialized but little longer than the leaves. In the plant, as in the animal, the organs present have a capacity above the ordinary requirements of life, and therefore senile degeneration in function can continue for some time before the point is reached at which the efficiency drops below the daily requirement. This interesting point will be considered again under the section dealing with carbohydrate production.

If meristematic tissue does undergo senile deterioriation, then cambium must produce less efficient conducting tissue as time goes on, and ultimately its efficiency will sink to a point at which the leaves will begin to suffer from lack of supplies as well as from their own senile degeneration, and senile death will come apace. In these experiments, however, the older vines, with four exceptions, were in the stage of vigorous maturity and evidently had not reached the stage of inefficient transport. The idea that the differences in size of vein islets is primarily due to difference in efficiency of transport in older parts of the plant must therefore be definitely abandoned.

The possibility of the production of toxic substances by the old parts of the root and the stem, and their being carried up to poison the meristem and the leaf, is a fascinating conception. In the theory of senility which Metchnikoff (1908) has based on the autotoxin conception, the source of the toxin is said to be in man the large intestine and in annual plants the flower; while in order to meet the conditions of the present problem with perennials, the old wood and bark must be considered the guilty organs.

It is possible that autolytic changes occur in the cells of the old specialized tissues of the wood and the bark with the production of various substances. It is possible also that some of these substances may escape from the region of their origin, enter the transpiration stream and be carried to buds and leaves, and accumulate there in sufficient concentration to affect the meristematic tissue. But there is no direct evidence that toxic substances are produced or that such poisoning occurs. On the contrary, the evidence from the studies of venation here described is directly opposed to the theory that the changes in size of the vein islets are due to contact with toxins produced from the old specialized tissues of the plant. In the mature leaves of *Vitis vulpina* growing under natural conditions, the evident luxuriance of growth and the healthful appearance of the leaves does not suggest the presence of toxins. Any toxin that would act solely on the venation of the leaf could do so only through mechanical obstruction of the vessels. Since any toxins that reach the leaves must be soluble and readily diffusible through protoplasm, such obstructive action is hardly possible.

The fact that the removal of cuttings from contact with the old vine and their subsequent growth on their own newly developed roots did not cause a distinct increase in size of the vein islets in their leaves, is a convincing demonstration of the inadequacy of the toxin theory. If leaves removed from contact with a suspected source of toxins show the same characters as those that remain in contact with it, then the absence of the suspected toxins is established. The possibility that a permanent poisoning may have been produced in the twig before it was removed from the vine as a cutting, is opposed to the universal recuperative power that all life possesses. The growth of cuttings on their own roots or on the roots of seedlings establishes the conditions needed for complete recuperation from the effect of any toxin so feeble as to cause no evident decrease in the vigor of growth, even when contact with continuous contamination is unbroken. The cuttings of large diameter which had several annual rings and had therefore been subjected to any possible toxic action three times as long as had cuttings from the same vine with one ring, produced leaves whose vein islets were of the same size as those in the smaller cuttings.

The idea may be dismissed, therefore, that conduction of toxic products from the stem into the leaves is the primary cause of the decrease in size of vein islets that comes with age. It may be true that there is later some effect of this kind, but it cannot be considered the primary cause. Of course, when the trunk is decaying or is suffering from the attacks of other organisms, the toxins produced are the results of external agencies and the case is one of disease, not senility. Even in such striking cases as those in which great hollows are formed by the decay of the heartwood, the leaves often seem to be suffering no toxic action.

It is possible that one of the accompaniments of senility is an increase of toxic products in the cells. But if such were the case, this accumulation would not be confined to any one class of cells, but would occur in all cells of the senile organism. The cells of the leaf would suffer from their own toxic products as the plant becomes senile, and so would the other active parts of the plant. The failing activity of each would then hasten the senile degeneration of the others, but would not be the primary cause.

It seems to be true, from the facts brought out in the foregoing discussion, that the change with age in the size of the vein islets in the leaves is not the result of any changes that have occurred in the old specialized tissues of the plant. The evidence demonstrates that neither decrease in the efficiency of excretion nor toxin production in the old specialized cells can account for the change in venation. There remains for investigation the possibility that the changes in size of vein islets may be due to the increasing size of the plant and the consequent change in the position of the leaves.

In the case of the paired vines of *Vitis vulpina*, the external conditions were much the same, and leaves were picked which had been growing under similar conditions of exposure to light. Care was taken also that the leaves selected were growing at the same height above the ground and at approximately equal distances from the bases of the vines. The leaves of the paired vines were therefore growing under much the same external conditions in both the young and the old vines. In spite of this fact they showed the differences in venation that have been tabulated above. Even more striking is the evidence from root shoots and water sprouts of various plants, the leaves of which, although borne close to the ground, possessed vein islets of a size typical of the age of the stump. The sizes of the vein islets in the leaves borne by cuttings raised under identical conditions furnish the final conclusive evidence that differences in external conditions are not the cause of the change of venation with age.

It may be concluded, therefore, that the progressive decrease in size of vein islets which accompanies the increasing age of the perennial plants examined is caused neither by changes in the older parts of the plant nor by the increasing size of the plant. The visible change in venation is therefore the result of a progressive change in the cells of the meristem and of the leaf which is inherent in the nature of their protoplasm. Such inherent progressive change in animal protoplasm is called senility. Animal senility is marked by an initial rapidity and later slowing of its onset, and may be represented by a curve which corresponds to the lower half of a parabola with the equation of the general nature of $(Y-A)^2 = BX$. In such a curve Y represents the condition of function or structure, X represents the age, and A and B are specific constants for the case in question.

By a comparison of the curve of decrease in size of vein islets and that of some recognized senile degeneration in animals, some evidence of the significance of the change in venation may be obtained. In figure 58 two such curves are shown, curve B representing the senile decrease in rapidity of growth of guinea pigs from birth to the age of three months, and curve A representing a decrease in size of vein islets in the leaves of *Vitis vulpina.* Both curves can be expressed by the same equation with different constants, and are essentially of the same type. Similarly, figure 57 (page 317) exhibits the common characters of a senile progressive change in an animal activity and the progressive change in venation in the leaves of cuttings.

The elimination of other possible causes of the change with age in the size of vein islets, and the marked similarity to typical senility in animals shown by the rate of its progress, is strong evidence that the real cause is senile degeneration of the meristematic tissue and of the cells of the leaf. That the increase with age of vascular tissue in the leaf is a degeneration as regards physiological activity will be made plain.

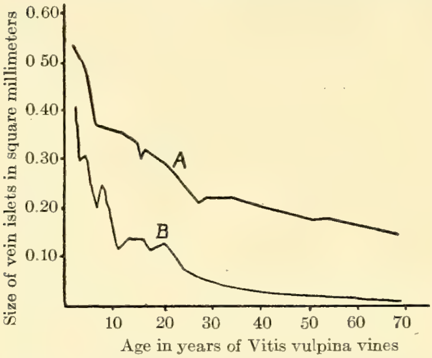

FIG. 58.—*Curves showing (A) senile decrease in size of vein islets in leaves of Vitis vulpina, and (B) senile decrease in rate of growth of guinea pigs*

COMPARISON OF PHOTOSYNTHETIC ACTIVITY IN LEAVES OF YOUNG AND OF OLD PLANTS

It has been shown by Schuster (1908) that the veinlets are of little or no importance in the mechanical support of the leaf. If, therefore, the increase in the proportion of vascular tissue and the consequent decrease in photosynthetic cells result in a decrease of photosynthetic activity, the change in venation with age is a loss without any compensation. Such a loss in photosynthetic activity must obviously accompany the decrease in photosynthetic cells unless the latter improve in efficiency as rapidly as they decrease in amount.

In animals the specialized cells persist for the entire length of life of the organism, and therefore an increase in efficiency of the specialized functions may occur. There are two principal causes for such an improvement, both resulting from repeated exercising of the special activity involved. The first is the improvement in concerted action between the different specialized tissues involved in even the simplest activity. The second is the increase in size and power of the specialized cells concerned, which not only continues as long as the original impetus of vital activity persists, but is stimulated by the mutual interaction of related tissues. The benefit of practice in athletics, for example, is due to improved coordination in action of muscles, nerves, heart, lungs, and kidneys, as well as to the increased specialized power that exercise stimulates in the cells of the tissues of these organs. In animals, therefore, the increase in efficiency is due to a longer period of interaction and development of specialized tissues.

The specialized tissues in the leaf of the old vine have neither worked longer together, nor had a longer period of development, than the specialized tissues in the leaf of the young vine, and therefore there is no possibility of any such increase in efficiency of any of their tissues as that which may occur in animals. Decrease in assimilating tissue means, therefore, a decrease in the amount of carbohydrate production by the leaf.

In order to get actual figures on this point a number of determinations were made of the increase in weight during daylight by the leaves of old and of young plants. As the leaves of the cuttings were reserved for another purpose, the leaves of the paired vines had to be used. Since in only a relatively few cases a sufficient number of leaves with identical exposure to light could be obtained, the determinations are too few to give more than a general corroboration of the reasoned effect of the change in venation. The impossibility of using gasometric methods on a wide range of wild vines justifies the use of a method that is not strictly accurate, but that is capable of giving at least relative results. The method used was to punch disks, with a Ganong leaf cutter, from both sides, the base, and the apex of each of a number of selected leaves on the paired vines in the morning, and again at night, carefully drying the disks for three days at 80° C., and determining the gain in weight as a percentage of the weight of the morning disks. Only relative results were sought for, and therefore no system for measuring light intensity or recording temperature was used. The disks were killed by steam immediately after punching, and the various standard precautions were observed in their manipulation. The results with the paired vines are shown in table 46:

TABLE 46. VINES WITH 6 AND 20 RINGS COMPARED AS TO INCREASE IN DRY WEIGHT IN LIGHT

	Number of disks	Morning weight (grams)	Evening weight (grams)	Gain (per cent)
Vines with 6 and 20 rings				
Vine with 6 rings	98	0.2244	0.2530	12.7
Vine with 20 rings	104	0.3099	0.3163	2.1
Vines with 20 and 40 rings				
Vine with 20 rings	95	0.3623	0.3956	9.2
Vine with 40 rings	95	0.2752	0.2996	8.9
Vines with 8 and 25 rings (The day on which this comparison was made was cloudy and dark)				
Vine with 8 rings	52	0.1520	0.1642	8.0
Vine with 25 rings	49	0.1912	0.1900	0.6
Vines with 6 and 20 rings				
Vine with 6 rings	35	0.1761	0.2024	14.9
Vine with 20 rings	35	0.1460	0.1560	6.8
Vines with 5 and 25 rings				
Vine with 5 rings	45	0.1230	0.1356	8.6
Vine with 25 rings	50	0.1394	0.1356	−2.0
Vines with 8 and 24 rings				
Vine with 8 rings	35	0.0832	0.0854	2.6
Vine with 24 rings	69	0.1844	0.1860	0.9

These determinations were made late in August, and are doubtless low in percentage of gain for all the vines as contrasted with carbohydrate formation earlier in the growing season. This does not militate against the results furnishing a fair comparison between the old and the young vines. In fact, it tends to make the test a more delicate one. On very dark days the leaves of the old vines were not able to produce as much carbohydrate as was used, while on the same days the leaves on the young vines adjacent were able to make at least a small gain.

The vines with 20 and 40 rings, used in the second experiment, are not included in the following summary, as both belong to the same class and no results from young vines on the same day were obtained from vines adjacent to them:

Average gain for leaves of vines with from 5 to 8 rings	9.4 per cent gain in weight
Average gain for leaves of vines with from 20 to 25 rings	1.4 per cent gain in weight

A comparison between the carbohydrate-producing power of leaves in different stages of development on vines of *Vitis vulpina* gave the following results:

Average gain for leaves from 2.5 centimeters long to mature, 6 rings	13.3 per cent
Average gain for leaves from 2.5 centimeters long to mature, 15 rings	9.2 per cent

A comparison between leaves of *Ampelopsis veitchii* eight years old and nineteen years old, growing on a south wall with as nearly identical light exposure as could be obtained by any means, gave average results as follows:

Average gain for leaves of vine eight years old	15.1 per cent
Average gain for leaves of vine nineteen years old	9.7 per cent

CONCLUSIONS REGARDING RELATIVE RATE OF CARBOHYDRATE PRODUCTION IN THE LEAVES OF YOUNG AND OF OLD VINES OF VITIS VULPINA AND AMPELOPSIS VEITCHII

While the difficulty of obtaining leaves with identical light relations on adjacent vines of different ages limited the experiments to but few in number, the unvarying result of the tests is strong evidence in support of the statement made above, that senile deterioration is occurring in the leaves of perennials. It is not probable that translocation is more rapid in the leaves of old plants than in those of young plants. The number of large veins is not increased, and the increase in number of veinlets probably only keeps pace with a decrease in the rate at which materials can diffuse through the cells of the leaf, as will be explained subsequently.

RATE OF ELIMINATION OF CARBON DIOXIDE IN PICKED LEAVES OF VITIS VULPINA OF DIFFERENT AGES

It seemed possible that a comparison of the rate at which carbon dioxide is eliminated from the leaves of old and of young vines would give some evidence of the relative katabolic activity of the two stages. Since as near the normal respiration as is possible in detached leaves was desired, care was taken not to subject the leaves to other disturbing conditions. The method was as follows: Ten healthy, mature leaves of each vine to be studied were picked and were taken at once to the laboratory. Here they were carefully wiped clean of dust with a soft, dry cloth, the petioles were cut off at the base of the blades, and the blades were weighed. Each set of ten leaves was then placed in an air-tight respiration chamber. The respiration chamber, a 4-liter bottle, was supplied with air which had been drawn in turn through a gas-washing bottle employed as a safety and through a gas-washing bottle filled with a concentrated solution of potassium hydrate. The air leaving the respiration chamber was passed through a calcium-chloride tower for the purpose of removing its water vapor, then through a potash bulb, then through another calcium-chloride tower, and finally through another safety. The air current was provided by means of an aspirator with a regulated outflow.

Eight series of this type were set up side by side in such a way that temperature conditions were the same for all. Tests of old and of young leaves were run simultaneously, and frequent blanks in the different series were employed as checks. The leaves were so arranged in the containers as to prevent their packing together and thus possibly giving rise to such heat production as Molisch (1908) describes. The containers were covered with black paper. The experiments were brought to a close as soon as any indication of injury to the leaves appeared.

In tables 47 to 51 the percentage given in the last column is based on the green weight of the leaf and the weight of the carbon dioxide collected in the potash bulbs and reduced to the average per hour for the entire time of the test. It represents, therefore, the hourly percentage of carbon dioxide eliminated from the green leaves. This rate will be lower than if the dry weight of the leaves could have been used. Since this could not be obtained before the experiment, and the leaves at the end of the test had undergone respiratory losses, the use of the green weight as the basis of calculation was necessary. The leaves from the younger vines contain a greater percentage of water than do those from the older ones; therefore the use of dry weight as the basis would show a still greater difference in favor of the rapidity of production of carbon dioxide in the younger vines than is evidenced in the following data, in which the green weight is used.

Weighings, and determinations of percentage of carbon dioxide eliminated, were made at the intervals stated in the tables, in which also the total percentage of carbon dioxide eliminated is given. This total percentage is figured as the average rate of elimination per hour for the entire period of the test.

TABLE 47. RATE OF ELIMINATION OF CARBON DIOXIDE FROM PICKED LEAVES OF VITIS VULPINA WITH 20, 6, AND 8 RINGS

Age (years)	Weight of ten leaves (grams)	Weight in grams of carbon dioxide eliminated in successive periods of				Total number of hours	Percentage of carbon dioxide per green weight per hour
		22½ hours	26½ hours	46¾ hours	23½ hours		
20	26.6	0.2744	0.0534	0.2374	0.0675	119¼	.020
6	33.3	0.6507	0.0665	0.3478	0.1986	119¼	.032
8	48.7	0.5662	0.1270	0.3904	0.4060	119¼	.026

TABLE 48. RATE OF ELIMINATION OF CARBON DIOXIDE FROM PICKED LEAVES OF VITIS VULPINA WITH 5, 6, 25, 8, 25, 20, AND 8 RINGS

Age (years)	Weight of ten leaves (grams)	Weight in grams of carbon dioxide eliminated in successive periods of			Total number of hours	Percentage of carbon dioxide per green weight per hour
		20 hours	23 hours	26 hours		
5	6.5	0.0778	0.0917	0.0678	69	0.052
6	7.4	0.0211	0.0408	0.1288	69	0.038
25	14.6	0.0870	0.2115	0.1765	69	0.047
8	20.0	0.0637	0.2456	0.2504	69	0.041
25	26.5	0.0076	0.2286	0.4424	69	0.037
20	12.0	0.0125	0.1819	0.1462	69	0.041
8	9.8	0.0092	0.2540	0.1262	69	0.057

TABLE 49. RATE OF ELIMINATION OF CARBON DIOXIDE FROM PICKED LEAVES OF VITIS VULPINA WITH 56, 27, 5, 8, AND 7 RINGS

Age (years)	Weight of ten leaves (grams)	Weight in grams of carbon dioxide eliminated in successive periods of			Total number of hours	Percentage of carbon dioxide per green weight per hour
		17½ hours	22 hours	5 hours		
56	8.2	0.0104	0.0584	0.0248	44½	0.026
27	17.0	0.0768	0.0900	0.0448	44½	0.028
5	7.1	0.0492	0.0382	0.0082	44½	0.030
8	18.0	0.0682	0.0584	0.0348	44½	0.020
7	9.3	0.1406	0.0778	0.0300	44½	0.060

TABLE 50. RATE OF ELIMINATION OF CARBON DIOXIDE FROM PICKED LEAVES OF VITIS VULPINA WITH 24, 8, 21, 7, 10, 10, 23, AND 5 RINGS

Age (years)	Weight of ten leaves (grams)	Weight in grams of carbon dioxide eliminated in successive periods of				Total number of hours	Percentage of carbon dioxide per green weight per hour
		19½ hours	24 hours	51 hours	20 hours		
24	22.1	0.0418	0.1334	0.2964	0.0506	114½	0.021
8	10.8	0.0262	0.0814	0.1212	0.0324	114½	0.021
21	30.5	0.0894	0.2812	0.5900	0.1363	114½	0.031
7	27.3	0.0502	0.1350	0.2932	0.0344	114½	0.016
10	15.0	0.0908	0.1548	0.3436	0.0840	114½	0.039
10	25.3	0.0586	0.1398	0.1891	0.0393	114½	0.015
23	14.4	0.0372	0.1009	0.0927	0.0396	114½	0.017
5	7.7	0.0203	0.0692	0.1008	0.0234	114½	0.025

TABLE 51. RATE OF ELIMINATION OF CARBON DIOXIDE FROM PICKED LEAVES OF VITIS VULPINA WITH 6, 9, 18, AND 20 RINGS

Age (years)	Weight of ten leaves (grams)	Weight in grams of carbon dioxide eliminated in successive periods of			Total number of hours	Percentage of carbon dioxide per green weight per hour
		5¼ hours	16¼ hours	32½ hours		
6	9.2	0.0468	0.0632	0.0932	54	0.041
9	15.3	0.0282	0.0606	0.1598	54	0.030
18	16.2	0.0645	0.0407	0.1938	54	0.034
20	11.2	0.0113	0.0354	0.0789	54	0.023

TABLE 52. SUMMARY OF RESULTS ON RATE OF ELIMINATION OF CARBON DIOXIDE FROM PICKED LEAVES OF VITIS VULPINA OF DIFFERENT AGES

Average age of young vines used	7.3 years
Average age of older vines used	25.4 years
Number of determinations made	92
Average length of each determination	23.86 hours
Rate of elimination of carbon dioxide in young vines	.0349 per cent per hour
Rate of elimination of carbon dioxide in older vines	.0297 per cent per hour

The general average for the plants tested indicates that the rate of elimination of carbon dioxide from the picked leaves was about 17 per cent more rapid in the young plants than in the older ones. Any function so complex as respiration is affected by many factors. While conditions were identical in these tests as regards external conditions, and while the results doubtless indicate a true difference in the metabolic power of the respective tissues, nevertheless irregularities are present which point to differences in some factor other than age. The amount of water present is certainly one of these differences.

The respiratory values here shown do not necessarily indicate a slower katabolic activity on the part of each individual cell of the leaf. This may or may not be the case. The total decrease for the leaves of the older plants is doubtless due in part to the lesser total volume of parenchymatous tissue in them as compared with that in the leaves of the younger plants. Whatever the cause of the slower respiration in the older leaves, its presence may indicate that the leaves of the younger vines are more efficient organs.

The evidence presented seems to show that the encroachment with age of vascular tissue on the space occupied by photosynthetic cells in the leaf of a young plant results in decreasing assimilation and respiration. The decrease in size of the vein islets is therefore a real degeneration in the structure of the leaf, just as the invasion of connective tissue into the higher tissues in senile animals is a degeneration. As the replacement of photosynthetic tissue by vascular cells progresses with age, each new crop of leaves will be less efficient in the production of carbohydrates than the preceding crop; and ultimately the plant will begin to suffer from lack of food, and if no other cause of death intervenes it will in time starve to death. The rapidity of the progress of senile degeneration in plants varies with the species, as it does in animals, but ultimately senile death will occur.

It is not possible at present to state the precise nature of the change in meristematic cells which forces the production of more vascular tissue at the expense of parenchymatous tissue. Later in this paper an attempt at an explanation will be made.

IMBIBITION OF WATER BY POWDERED LEAVES OF YOUNG AND OF OLD VINES

The amount of water imbibed by the powdered tissues of leaves gives an indication only of the relative water-holding capacity of the living leaves. Is there any difference in the behavior of leaves from young and from old vines toward water of imbibition?

A large number of leaves were collected from various vines of *Vitis vulpina* with from six to eight rings and from twenty to twenty-five rings. These leaves were ground fine in a meat grinder. Each ground mass was immersed in acetone for ten minutes, filtered under suction, and dried in a crucible for three days at a temperature of 90° C. The dried masses were then thoroughly pulverized in a mortar to the same degree of fineness, the heavier veins and the more numerous veinlets of the older material requiring more grinding than the younger. There were thus obtained about 100 grams of leaf powder from the younger vines and the same quantity from the older ones. The individual vines were not kept separate but all those of similar age were mixed together.

DEGREE OF IMBIBITION WITH DISTILLED WATER

In order to determine the degree of imbibition of water by powdered leaves from young and from old vines the procedure adopted was as follows: In three graduated glass tubes was placed 0.5 gram of the powder from leaves of young vines, and into three other tubes the same quantity of powder from leaves of old vines. Distilled water was added to each. The material from young vines increased in bulk from a height of 30 millimeters to one of 50 millimeters, while that from old vines increased only from 25 to 38 millimeters; the percentage increase of the leaves from the young vines being 66, and that of the leaves from the old vines being only 52.

This result proved to be a constant one, there being minor variations in the percentages, but the powder from the young leaves always averaging about 14 per cent greater absorption. This difference might be due to a greater power of imbibition in the cellulose and protoplasm of the young tissues, or, as Fischer (1910) has shown, to a greater degree of acidity in the younger cells. That Fischer's results for animal colloids hold, to some degree at least, for plant colloids, is indicated by the following test: In the bottom of each of six graduated glass tubes was placed 0.2 cubic centimeter of leaf powder from the old leaves, and into each tube was poured 2 cubic centimeters of distilled water or of the concentrations of oxalic acid indicated below. The concentration of oxalic acid is expressed in terms of gram-molecular solution. The results are shown in table 53:

TABLE 53. EFFECT OF DIFFERENT CONCENTRATIONS OF OXALIC ACID ON ABSORPTION OF WATER BY POWDERED LEAVES

Test tube	Added 2 cubic centimeters of	Time (hours)	Height of column of swollen powder (centimeters)
A	Distilled water	24	2.8
B	0.005 oxalic acid	24	3.8
C	0.02 oxalic acid	24	4.5
D	0.1 oxalic acid	24	3.8
E	0.5 oxalic acid	24	3.3
F	Distilled water	24	2.7

The swelling was greater in the weak acids than with distilled water, and reached a maximum at 0.02 molecular solution of oxalic acid.

The fact that the powder from the young leaves swells more than that from the old leaves may therefore be due to the fact that the leaves of the young vines are richer in some organic acid than are those of the old vines.

RELATIVE ACIDITY OF THE YOUNG AND THE OLD POWDERED LEAVES

In each test tube 0.2 gram of powder was placed, 2 cubic centimeters of distilled water was added, and the test tube was shaken. The acidity of the water was then neutralized by titrating with 0.1 normal KOH, phenolphthalein being used as an indicator. The powder does not in any way interfere with the use of this indicator.

TABLE 54. RELATIVE ACIDITY OF LEAF POWDER FROM YOUNG AND FROM OLD VINES

Amount (in cubic centimeters) of 0.1 normal KOH solution required to neutralize 0.2 gram of			
	Powder of young leaves		Powder of old leaves
	2.8		1.4
	2.8		1.6
	2.5		1.6
	2.0		1.6
	2.0		1.6
	2.0		1.6
	2.0		1.3
	1.9		1.3
Average..........	2.2	Average.......	1.5

The powder from the leaves of the young vines, therefore, has a greater degree of acidity than that from the leaves of the old plants. This is a distinct difference between the leaves of plants of different ages. The significance of this difference as regards physiological activity cannot be certainly indicated. It may affect the degree of turgidity and the permeability of the cells of the leaves.

IMBIBITION AFTER NEUTRALIZATION

In order to see whether the greater acidity was the sole cause of the greater swelling, experiments were made in first neutralizing the solution, then washing it, and then adding 0.02 molecular solution of oxalic acid and noting whether differences in swelling still appeared. In these experiments 0.2 gram of leaf powder was placed in each test tube and 2 cubic centimeters of distilled water was added. Each solution was then neutralized with 0.1 normal KOH and washed. Two cubic centimeters of 0.02 molecular solution of oxalic acid was then added, with a trace of thymol, and the tubes were left for twenty-four hours. The wet powder, after being gravity-drained for five minutes in the inverted test tube, was weighed and the percentage of imbibed water was calculated as a percentage of the weight of the dry powder. The results are shown in table 55:

TABLE 55. Amount of Water Imbibed by Leaf Powder from Vines with from 6 to 8 and from 20 to 25 Rings

	KOH (cubic centimeters)	Oxalic acid (2 grams of)	Height of column (millimeters)	Weight of imbibed water (grams)	Percentage of imbibed water
Young leaves					
1	2.00	.02 mol. sol.	53	0.9968	498.4
2	2.00	.02 mol. sol.	53	0.9298	464.9
3	1.95	.02 mol. sol.	54	1.0018	535.9
Old leaves					
1	1.40	.02 mol. sol.	48	0.9458	472.9
2	1.35	.02 mol. sol.	46	0.7948	397.4
3	1.30	.02 mol. sol.	42	0.8168	408.4
Young leaves					
1	2.80	.02 mol. sol.	....	0.9588	474.4
2	2.80	.02 mol. sol.	....	0.9852	479.1
3	2.50	.02 mol. sol.	....	0.9847	492.4
4	2.00	.02 mol. sol	....	1.0194	509.7
5	2.00	.02 mol. sol.	....	0.9141	457.1
Old leaves					
1	1.60	.02 mol. sol.	....	0.6076	303.8
2	1.60	.02 mol. sol.	....	0.6692	339.6
3	1.60	.02 mol. sol.	....	0.7512	375.6
4	1.60	.02 mol. sol.	....	0.5875	293.8
5	1.60	.02 mol. sol.	....	0.8054	402.7
6	1.60	.02 mol. sol.	....	0.9938	496.9

General average of percentage of imbibed water for young leaves	489.0 per cent
General average of percentage of imbibed water for old leaves	387.9 per cent

The substance of the younger leaves has a greater inherent property of imbibing water than that of the older leaves. Some of this difference, no doubt, is due to the greater proportion of vascular tissue present in the older leaves, but the difference in percentage of imbibition seems to be greater than would be caused by the difference of vascular tissue. This greater capacity of imbibing water, if present in living leaves, must play a part in the distribution of substances among the cells, as well as directly affect other activities of the protoplasm.

OTHER EVIDENCES OF SENILITY

The investigations on veinage, photosynthetic activity, respiration, and imbibition point to the conclusion that leaves of old vines have suffered senile degeneration and that they are less efficient organs than leaves of young plants. Since such a very marked character as veinage is altered by age, it is probable that other differences exist between leaves of young and of old vines. A certain number of observations made indicate that there are other structural and cell differences, and, while the evidence is not fully conclusive, it is at least suggestive and tends to further support the idea that senile changes occur in the leaves of Vitis. The observations are herewith recorded.

COMPARISON OF NUMBERS OF STOMATA IN LEAVES OF YOUNG AND OF OLD VINES OF VITIS VULPINA

Comparisons of numbers of stomata per square millimeter were made with leaves borne by cuttings grown in distilled water under identical greenhouse conditions. Thin flakes of epidermis were removed with a very sharp razor from different leaves of different cuttings, were mounted in water, and were measured as to the number of stomata. From each flake a vein islet of rectangular form was selected, its area measured, the number of stomata counted, and the number to the square millimeter calculated. The results are shown in table 56:

TABLE 56. COMPARISON OF NUMBER OF STOMATA IN LEAVES OF VITIS VULPINA

	Number of stomata to 1 square millimeter in		
	6 vines with from 5 to 7 rings	4 vines with from 8 to 10 rings	6 vines with from 20 to 30 rings
	82	105	247
	100	128	314
	133	138	288
	115	112	266
	115	125	266
	112	120	246
	165	150	266
	96	125	292
	119	155	300
	107	....	290
	112	....	330
	157	....	256
	115	....	314
	124	....	314
	144	....	247
	86	....	....
	105	....	....
Average..........	117	129	282

It has been shown by Mer (1886), by Dufour (1886), and by Schuster (1908) that the same species of leaf possesses more stomata to a unit of surface when growing in strong light than when growing in the shade, and Brenner (1902) has stated that at first, when leaves are moved into the shade, the increase in their size due to greater humidity causes the stomata to become farther apart, but that later new *Anlagen* are formed and the number is brought back almost to that found in sun-lit leaves. The differences in number of stomata per square millimeter on the leaves of *Vitis vulpina* given above, however, cannot be due to any difference in light exposure or humidity or available water, since the leaves used were borne on cuttings grown under identical conditions. It was noted also that the average size of the apertures of the stomata decreases with age, as does also the size of the guard cells. The figures given in table 57 were obtained from accurate measurements made on the same material as was used for counting stomata. Since the width of the apertures varies directly with the degree to which the stomata are open, while the length of the apertures is approximately the same at all times, the measurement selected as best representing the size of the stomata was the long diameter of the stomatal opening between the guard cells. Measurements of the width of the apertures of stomata when fully extended indicated that the relation of the long to the short diameter was much the same in both young and old vines. The long diameters of the stomata can therefore be used as a measure of the size of the apertures.

TABLE 57. SIZE OF STOMATA IN VITIS VULPINA OF DIFFERENT AGES

Long diameter of aperture in	
6 vines with from 5 to 7 rings (microns)	6 vines with from 20 to 30 rings (microns)
18.3	9.2
14.6	9.2
14.6	11.0
18.3	12.8
18.3	9.2
14.6	9.2
18.3	11.0
14.6	12.8
22.0	9.2
18.3	11.0
14.6	12.8
14.6	9.2
18.3	9.2
14.6	11.0
14.6	14.6
Average......... 16.6	Average........ 10.8

COMPARISON OF SIZES OF PALISADE CELLS IN LEAVES OF YOUNG AND OF OLD VINES OF VITIS VULPINA

The cells of the palisade layer are much more regular in size and form than any others in the leaf, and therefore best adapted for the determination of the effect of increasing age on the size of the cells of the leaf. In table 58 the measurement given is the length of the cell measured perpendicularly to the surface of the leaf, as seen in cross section.

TABLE 58. SIZE OF CELLS OF THE PALISADE LAYER OF LEAVES OF YOUNG AND OF OLD CUTTINGS OF VITIS VULPINA

	For 3 vines with 5 rings (microns)		For 3 vines with 20 rings (microns)
	14.6		11.0
	11.9		11.0
	11.9		11.0
	11.9		9.0
	12.8		11.0
	11.9		14.6
	12.1		10.0
	11.9		11.0
	11.0		9.0
	12.0		9.0
	11.0		9.0
	14.6		9.0
	11.0		9.0
Average.........	12.2	Average.........	10.3

The cells of the palisade layer thus show a distinct decrease in size even in the leaves of the plants twenty years old, which are in their vigorous maturity. The guard cells of the stomata, in the cases observed, also show the same change in size with age. This change in size shown by the two most regular cells of the leaf is seemingly apparent in all the photosynthetic cells as well. As regards the cells of the veinlets, this decrease in size with age is doubtful and will require more study. It is possible that the physiological stimulus which calls forth an increasing development of vascular tissue in the older plants may likewise so stimulate the development of the vascular cells as to neutralize, for a time, the tendency toward a reduced size. Some evidence to this effect has been observed.

These few observations indicate that possibly there is a difference in number of stomata in the leaves of young and of old vines, together with changes in guard cells and palisade cells. The results are not conclusive enough to permit of definite conclusions, and the inadequacy of the evidence does not warrant any explanation of its significance. Suffice it to say that an increased number of stomata with smaller apertures, while not permitting greater diffusion of carbon dioxide or oxygen than the stomata of young leaves, would be a benefit to the plant in bringing a larger area of palisade cells in closer proximity with air chambers. If the permeability is decreased in the cells of leaves of old vines, then a larger number of stomata per unit area would tend to counterbalance the defect of decreased permeability. The stimulus to the production of an increased number of stomata cannot be otherwise explained at the present time.

RELATIVE MASS OF NUCLEUS AND CYTOPLASM IN CELLS OF THE LEAF IN YOUNG AND IN OLD VINES OF VITIS VULPINA

It will be remembered that the advance of senility in animal cells is marked by a decrease in the relative amount of nuclear material as compared with the amount of cytoplasm. Difficulty was experienced in making such a comparison in the cells of the leaf, because of individual differences in the sizes of the nuclei of adjacent cells. The rapidity with which a cell is working is indicated usually by temporary changes in the size of its nucleus. The cells in the assimilating tissues of the leaf seemed to be in different stages in each preparation, and the differences were great enough to render doubtful the value of averages. The cells that did show some uniformity in this respect were the border parenchyma cells of the veinlets, and a series of measurements was made of these with the following results:

Proportion of cytoplasm to nucleus in vine with from 5 to 7 rings, 388 to 1
Proportion of cytoplasm to nucleus in vine with from 20 to 30 rings, 478 to 1

The great difference in the lengths of these cells makes the calculations, even in the border parenchyma cells, unsatisfactory. This comparison between nuclear and cytoplasmic material can probably be carried out with greater accuracy on the meristematic tissue in the stem. In the investigation of the effect of age on the tissues of the stem, which will be the next phase of the problem attacked, a careful study of the proportion of cytoplasm to nucleus will be made.

SUMMARY

1. The size of vein islets in the leaves of *Vitis vulpina* is independent of the area and thickness of the leaves, and is influenced only slightly by differences of exposure to light.

2. The vein islets in the leaves of *Vitis vulpina* become smaller as the vine becomes older. This decrease in size of vein islets is due to encroachment of vascular tissue.

3. Leaves borne on plants vegetatively reproduced show vein islets similar to the plant from which the original cuttings were secured. Only plants reproduced sexually show vein islets larger than those of the parents.

4. The same difference with age in the size of vein islets occurs in *Vitis bicolor*, and apparently in a considerable number of other woody perennials.

5. The decrease in size of vein islets means reduction in number of photosynthesizing cells, and the evidence shows that for leaves of old vines there is a decrease in the rate of photosynthesis.

6. There is also a decrease in the rate of respiration (evolution of carbon dioxide) in picked leaves.

7. Leaves of young vines have a greater capacity for imbibing water than have leaves of old vines, which may be the result of less vascular tissue in the former.

8. There is probably an increase with age in the number of stomata per square millimeter.

9. There is probably a decrease with age in size of stomatal aperture and of guard cells.

10. There is probably a decrease with age in size of palisade cells.

11. There is probably a decrease with age in the mass of nuclei of border parenchyma cells.

DISCUSSION

It is believed that the evidence presented is fairly conclusive that the changes discussed are due neither to external factors nor to internal factors other than those produced in the meristematic tissues by age, and that they must therefore be ascribed to the action of true senile degeneration of the type found in animals. The positive evidence that these changes are senile ones rests on three fundamental similarities which they show to senile degenerations occurring in animals: the first marked similarity is that the rate of change, as shown by tables and curves, is strikingly similar; the second is the fact that such physiological activities as are common to both plants and animals — respiration, for example — show the same changes with age; the third is the nature of the morphological changes. Although the structure of these plants is so different from that of animals that detailed comparisons in but few specific organs can be made, nevertheless the recognized theories of the causes of the senile changes in animal structure, if applied to plant organs, would call for such morphological degenerations as have been shown in this paper to exist in the leaves. Since these theories are given later, their application to this point will not be repeated here. In the case of senile changes in cells, while the proportion between the mass of nucleus and that of cytoplasm in young and in old leaf cells of *Vitis vulpina* was not determined without possible error, the considerable change in the proportion found with age seems too great to be ignored. The nature of the change in nuclear mass in these leaf cells is the same as that found in animal cells.

When considered in its entirety, the evidence that these changes are visible expressions of senile degeneration in the meristematic protoplasm seems to be reasonably substantial. At any rate, any other explanation of these changes in the leaf would be much more difficult to maintain.

If this be granted, it will be interesting to apply these tests of age to the long-disputed problem of the effect of continued vegetative propagation as compared with that of seed propagation. Heretofore no evidence of senility could be used that was not open to the objection that it might be merely the effect of some external agency.

SIGNIFICANCE OF SENILE CHANGES TO THE PROBLEM OF THE RUNNING-OUT OF VEGETATIVELY PROPAGATED FRUITS

The first publication of importance regarding the possibility of senile deterioration was that by Thomas Andrew Knight (1795), who attributed to this cause the gradual failure of different varieties of apples and pears. Having tried experiments in grafting scions from young and from old seed-grown trees of different ages, Knight decided that the cuttings were directly influenced by the age of the plant from which they were taken. He concluded that a variety propagated by cuttings would not be vigorous much beyond the age of the parent tree. He turns to nature for a most interesting support of his theory regarding the inherent defect of vegetative reproduction. Certain trees, he says, such as the aspen and some others, send up a multitude of shoots from the roots, while most of the wild trees are limited to seed propagation; and he adds, "Were a tree capable of affording an eternal succession of healthy plants from its roots, I think our woods must have been wholly over-run with those species of trees which propagate in this manner, as those scions from the roots always grow in the first three or four years with much greater rapidity than seedling plants."

In 1810 Knight published a paper entitled *On the Parts of Trees Primarily Impaired by Age*, in which he makes the following statement: "I am, therefore, much disposed to attribute the diseases and debility of old age in trees, to an inability to produce leaves, which can efficiently execute their natural office . . . . It is true that the leaves are annually reproduced, and therefore annually new; but there is, I conceive, a very essential difference between the new leaves of an old, and of a young variety."

Following Knight's first paper (1795) many observations which agreed or disagreed with his results were reported in English and Continental horticultural and gardening publications. None of these reports were more than casual notes.

The first paper of interest after Knight's was that by Jessen (1855), who strongly supported Knight's theory and introduced the term *sorte* to include all the plants grown from the cuttings of a parent plant. He believed that all the members of a *sorte* would grow old together.

In some degree the results of Bouche and Bolle (1875) supported Knight in the case of the cultivated olive tree.

The first paper to make a serious attack on the theory was that published by Sorauer in 1877. Sorauer maintained that cuttings showed the same type of differences among themselves that seedlings did, and might serve the same purpose without producing serious degeneration.

In 1882 Hildebrand published a long discussion on the duration of life in plants and the effect of various external factors in increasing or decreasing to some degree the constitutional duration. While this paper is full of interesting facts regarding this point, it nowhere touches the problem of senility as affecting constitutional duration, and therefore Hildebrand's results do not directly concern the present study.

Burgerstein (1895) made some observations on the relation of vegetative propagation to degeneration, arguing against its possibility.

The best review of the whole question is by Möbius (1897), who cites at considerable length the various observations on the conditions of those cultivated plants of to-day which have long been vegetatively propagated. His conclusion is that no senile deterioration in such plants has been proved to exist. He maintains that meristematic tissue is true embryonic tissue in the same sense as is the tissue in the embryo sac, and that there is no theoretical reason why propagation by cuttings should be different in its results from that by seeds. He believes that it has been shown by various published accounts that the banana, the grape, the date palm, the fig, and the Chinese yam (*Dioscorea batatas*), have been propagated for from two thousand to four thousand years by means of vegetative methods without degeneration. The number of writers which he cites is very large and could be greatly increased, but practically none of these reports can be considered as offering evidence of value.

It is evident, therefore, that the problem is still unsolved. It must be remembered that the lowest plants known to-day are apparently sexless, and capable of vegetative reproduction only. There can be little doubt of their ability to propagate their kind continuously over long periods of time. Only in those plants and animals which have developed sexuality and sexual reproduction, can the question of possible degeneration through long-continued vegetative propagation be seriously raised. Undoubtedly sexual reproduction efficiently rejuvenates protoplasm. Apparently it is also true that rejuvenescence is accomplished in the primitive sexless plants by an equally efficient method. In those higher plants that have lost their sexuality, it is possible that the loss of sexuality has been accompanied by the regaining of a more primitive method of rejuvenescence. It is in the seed-producing cultivated plants, the varieties of which are vegetatively propagated, that the problem of the relative efficiency of the two methods in rejuvenating protoplasm is presented with the least degree of complication.

First, therefore, such cultivated plants as the grape and the date should be dealt with, and later the more complicated cases, such as the Dioscorea. The records of the cultivation of the seed-producing fruits are not sufficiently complete to assure beyond question that the varieties which have been so long cultivated have not been replaced at intervals by seedlings. To-day, for example, countless seedlings of Concord grapes are being grown. Some of these develop into plants almost identical in vine and fruit with the original Concord. It is not beyond the bounds of possibility that some grape grower might confuse such a seed-produced vine with a true Concord graft. The younger vine might be utilized as a source for cuttings, and these in time might displace the older stock without any one's being the wiser. The possiblity of such propagation was greater in the past than it is to-day, and holds for every seed-producing cultivated plant. It is possible, therefore, that some of the seed-producing plants which are cited as conclusive evidence of the harmlessness of long-continued vegetative propagation have been sexually propagated at intervals.

The recognition of degeneration in cultivated varieties is rendered difficult by the very long life attained by woody perennials as compared with the life of man. Many trees have a natural life spanning several generations of men. Such plants would show little change in a single generation. The possibility of using the data gathered by the preceding generation as a test of deterioration of fruits to be utilized by following generations has been practically impossible in the past. The only criteria of degeneration known in the past have been such characters as yield of fruit and resistance to disease—in other words, characters that are profoundly affected by external conditions as well as by internal ones. It is evident that such characters cannot enable the growers of one generation to accurately compare the past and the present conditions of any varieties. Since investigators of to-day cannot make a mathematical summation of the combined effects of all the external factors that are operating throughout a season on a given plant, the inherent weakness of past comparisons needs no further comment. And yet all the arguments concerning the problem of the effect of vegetative propagation have been based on such comparisons and on theoretical considerations.

The chief theoretical assumption opposing the idea of degeneration through long-continued vegetative propagation in cultivated fruits has been that of the immunity of meristematic tissue to senile changes. The results of the present study of the senile changes in the leaves of *Vitis vulpina* argue against the truth of this assumption. What is of greater importance, in its practical application, is the discovery of specific morphological changes in the leaves as the plant grows old, which appear to be independent of external conditions and therefore available for use as tests of the physiological condition of the plant. It is hoped that the change in venation will prove in practice to be applicable to a wide range of cultivated plants as a practical and convenient method for the determination of the degree to which senile degeneration is present in any specific plant or variety of fruit. Whether it is also applicable as a test for the effect of apogamy has not been investigated.

While the purposes of this paper do not include the application of its findings to vegetatively propagated cultivated fruits, a few examinations of the venation of the leaves of certain varieties were made as preliminary tests and the results are given in table 59. The results obtained in the case of six varieties of grapes have already been stated, but for convenience will be repeated in the table. All plants examined were pedigreed plants growing in the orchards of the New York State Experiment Station at Geneva, New York, to which the writer had access through the kindness of Dr. Hedrick and his assistants.

TABLE 59. SIZE OF VEIN ISLETS IN SEVERAL VARIETIES OF FRUITS PROPAGATED BY CUTTINGS

Variety	Number of years since origin	Age of plant examined (years)	Number of intersecting veinlets in 2 centimeters
Concord grape	70	14	66
Worden (Concord seedling)	50	14	58
Concord seedling	7	7	35
Catawba grape	114	14	82
Diana (Catawba seedling)	63	14	74
Diana seedling	5	5	38
Lady apple	300	18	49+
Baldwin apple	92	18	41
Hubbardston apple	82	18	45
Hubbardston seedling	15	15	30
Seckel pear	100	20	54+
Seckel seedling	7	7	38
St. Catherine plum	215	10	52
Italian Prune plum	100	10	48
Green Gage plum seedling	15	15	33
Jefferson plum seedling	2	2	16
Red Cheek (Red Cheek Melocoton) peach	100	8	85+
Late Crawford peach	50	8	53
Elberta peach	25	8	41

In the cases of the Seckel pear and the Red Cheek peach the veinlets were too intricately branched to be accurately counted.

The leaves used for these data were picked at approximately the same height from the ground and care was taken that all had the same light exposure; so that conditions in each were as nearly alike as possible. The soil and the cultivation were apparently the same for each. Ten leaves were picked from each tree and the venation count given in the table is the average of the ten. At the time the venation count was made the writer did not know the respective ages of the varieties. An arrangement of the varieties in the order of their venation count proved an accurate forecast of the respective varietal ages as subsequently learned.

In so far as this preliminary test goes, therefore, it supports the view that propagation by cuttings does not prevent the progress of senile degeneration in the tissue of the cuttings. A number of interesting points suggest themselves on examination of table 59. In the case of the peach, probably the shortest-lived fruit represented in the table, it seems significant that the rate of decrease in size of vein islets is more rapid than in any of the others. Some species seem to have an original size of vein islets greater than closely related varieties. It is possible that such an initial large size of islet is a mark of physiological vigor, and indicates greater constitutional strength and a longer productive life than is the case with those plants with smaller islets. This point might well be considered by plant breeders in selecting breeding strains. On the other hand, some varieties—the grape, for example—seem to show a marked slowing of the rate of decrease in size of islets after maturity, and thus attain a considerable age in spite of the rather small initial size of the islets.

The possibility of determining the age of a plant by the size of the vein islets in the leaves opens the way to a comprehensive determination of the present physiological condition of the different varieties of fruits. The results obtained from the study of *Vitis vulpina* and the preliminary tests of cultivated fruits seem to indicate that all varieties of fruits now vegetatively propagated must in time run out. A wise provision for the future of the fruit industry must therefore include an investigation into the physiological condition, as regards senile degeneration, of each variety. Such a study may show some varieties to be capable of some degree of rejuvenescence through processes similar to those found in sexless plants, or it may show that all are doomed to extinction. Certainly, the determination of the fact is important. If certain varieties must shortly show deterioration, plant breeders must pay particular attention to developing seedlings capable of replacing them. Such seedlings having been developed, their propagation by cuttings may then be continued until physiological deterioration again appears and recourse to a new seedling variety is necessary. It may thus be possible to so classify and control cultivated fruits that all loss from degeneration may be accurately foreseen and prevented.

Before leaving the subject of the effect of vegetative propagation on different varieties, it may be well to point out that in those cases in which senile degeneration has progressed so far that the conduction of nutritive materials to the leaves is markedly interfered with, the removal of a scion and its insertion into a seedling stock should result in an improvement in its nutritive supply. This may produce a certain increase in activity, but not rejuvenescence since the cells of the scion are not changed.

The effect of vegetative propagation on plants other than fruits, potatoes, hops, and the like, is also of great importance. The general opinion among growers seems to be that those varieties which have been long propagated in this way are showing decrease in yield and in resistance to disease. No preliminary applications of the venation method have been made in the case of these plants. Where such evident adaptations for vegetative propagation as tubers, bulbils, and the like are present in plants, the possibility of the presence of other than sexual methods of rejuvenescence seems greater than in plants without such special equipment. An examination of the leaves borne by trees and root shoots of Lombardy poplar (*Populus nigra italica* Du Roi) — which has lost its power to produce seeds but sends up multitudes of root shoots, and which has been propagated artificially by cuttings for about seven hundred years — showed the vein islets to be so divided by minute veinlets of procambial tissue as to make measurement very difficult. The application of the venation test to other cultivated plants vegetatively propagated should give interesting results and will be undertaken.

BEARING OF THESE FACTS ON THEORIES OF SENILITY

The steady encroachment with age of the vascular tissue of the leaf on the space originally occupied by the photosynthetic cells, and the accompanying decrease in the photosynthetic power of the leaf, must be forced by some physical or chemical change occurring in the cells.

In other words, it must be a visible effect of a progressive senile change occurring in the meristematic cells which produce the leaf.

An examination of the vein islets under the microscope shows that a majority of the photosynthetic cells are not in direct contact with the veinlets. Such cells must get their water and inorganic nutrients by osmosis and diffusion through the cells which separate them from the veinlets, and all outgoing materials must also pass through the enclosing cells. The larger the vein islet, therefore, the greater will be the average distance through which materials must be conducted before they reach the veinlets or the cells. It is obvious that the maintenance of efficient ingress and egress is vital to the life of the cells and the life of the plant. Since most of the cells are dependent on the ability of surrounding cells to conduct materials, the process of diffusion through photosynthetic cells is of vital importance to the whole plant.

The results of Askenasy (1870), Volkens (1884), Massart (1902), and Schuster (1908) indicate that if the cells of the leaves are suffering for water additional veinlets are produced. In other words, the development of veinlets is in part controlled by the condition of the cells supplied by them. If the photosynthetic cells are suffering from the lack of something which the veinlets can supply, the effect is to stimulate the development of additional veinlets. An interesting theory of the origin of the small blood vessels in the developing animal embryo, advanced by Mühlmann (1900), is based essentially on the same idea. As each aggregate of cells in a developing embryo increases in size, its central cells will be pushed farther away from the blood vessels at its margin. Finally this distance becomes so great that osmosis and diffusion through the intervening cells cannot efficiently provide for the needs of the central cells. This lack of efficient nutrition and excretion causes certain modifications in the cellular activities. These specific modifications stimulate the formation of branches from the original blood vessels, which penetrate the cell aggregate. The entrance of the blood vessel is physiologically the equivalent of a reduction in the size of the aggregate.

In the leaf of the young plant, the vein islets are as large as will permit efficient conduction to and from the central cells. There is no further increase in size. But if the protoplasm of the cells of the aggregates changes with age in such a way that osmosis and diffusion through them are being rendered increasingly difficult, the physiological effect will be equivalent to an increase in the distance from the veinlets. If one effect of age on the plant protoplasm is to decrease its permeability to any or all of the substances that pass in and out of the cell, the effect on the central cells of the vein islets will be essentially the same as removing them further from the veinlets.

On the assumption that such a decrease in permeability is one of the marks of senile degeneration occurring in plant cells, the differences between the sizes of vein islets, the number of stomata, the size of the cells, and the physiological activities, found between the leaves of young and of old plants, can be rather plausibly explained. The smaller size of the cells with age causes an additional interference with the freedom of osmosis; but this, it is believed, is secondary to the change in permeability, and probably is a result of it. The evidence given here of a possible decrease in permeability as a senile change in protoplasm, suggests its application to the theories of senility.

THEORIES OF SENILITY

The theories of senility may be grouped in two general classes: (1) those considering the cause to be failure of some specific organ in the body, and (2) those considering the cause to be specialization of the cells or change in the physiological activities of the cells. The theories in the first class cannot be considered as fundamental.

Localization of senile changes

Demange (1886) and Osler (1892) considered arterial sclerosis as the cause of senility, while Lorand (1912) ascribed senility to a degeneration of the thyroid and other ductless glands. Metchnikoff (1903 and 1908) explained senile degeneration in man as due to the action of intestinal bacterial toxins, and in plants to the formation of poison in the flowering shoot.

Metchnikoff's theory of the poisoning of the body by toxins produced in one part of it represents merely a different phase of the " guilty-organ " class of theories. He thinks that it is the large intestine in man, and the flowering shoots in the higher plants, that serve as centers of poison production which ultimately poisons the whole organism. In animals he considers the poison to be produced by bacteria in the intestine. The toxins thus formed are carried to all parts of the body by the blood, and attack various tissues with different degrees of success. Those cells that cannot successfully withstand the effects of the poison are weakened and destroyed by the independent predatory cells of the body — the phagocytes. Those plants that die soon after producing their seeds, Metchnikoff believes to have been poisoned by poisons formed in the flowers during seed production, which spread from the flowers to all parts of the plant and kill it.

Here again the theory is based on the conception that but one part is the cause of senility — that in spite of the fact that all the cells have a common origin in the development of the animal or the plant, only those of one organ are the producers of senility. As regards both plants and animals, Metchnikoff's theory is obviously of very limited application, being inapplicable to animals without the large intestine and to flowerless plants. As regards flowering plants, moreover, the enormous span of life attained by those that produce annually myriads of flowers and seeds is sufficient to eliminate further consideration of the poisoning theory as of primary value.

As regards animals, furthermore, Metchnikoff's theory is not so much a theory of senility as a denial of senility. In so far as he considers the cause of natural death to result from toxins produced by bacteria, he is denying the existence of natural death. In all bacterial diseases their toxins are the most important factors in the production of harm, whether they are localized in the intestine or in the tissues, and when death results from bacterial toxins it is due to disease, not to senility.

Disease is an interference with the vital activities, produced by unfavorable conditions — such as attacks of microscopic organisms inherited or acquired, structural defects in one or another organ, or the like. Senility, on the other hand, produces an inevitable death in spite of the most favorable external conditions and when all organs are sound. It has been shown that in senile degeneration all tissues have undergone a structural and physiological deterioration, and therefore all organs are involved.

Attempts to single out one particular organ as the sole agency of senility are therefore not supported by evidence and are based on a superficial conception of the process.

Cell specialization

As the seed or the egg develops into the mature organism, the embryonic cells gradually develop the typical cytoplasmic characteristics that mark the adult tissues. The cytoplasm not only increases in amount relative to the nucleus, but becomes structurally differentiated as well, both changes being of such a nature as to fit the cell for the efficient performance of the special work that it is to perform for the whole organism.

Minot, in 1891, was the first to advance the theory that this specialization of the cell was the cause of senility. He maintained at that time and later (1908) that the advance in efficiency in the function utilized by the organism was at the expense of the functions necessary to the continuous vigor of the cell. According to his view, therefore, each advance in efficiency of a special function was at the cost of deterioration in the self-sustaining power of the cell, and this deterioration he considered to be the true senile degeneration, which progressed to a final natural death.

Hertwig (1906) agrees with Minot's theory to this extent, expressing his idea in his own terms. He says that a free cell is completely free to carry on the activities required in order to maintain the vigor of the cell, and these activities he includes in the term *cytotypical.* The cells of the embryo, while largely under the cytotypical laws, are to some extent modified in their actions by the *organotypical* laws, which govern the interactions of the cells in each many-celled organism. As development proceeds, the cytotypical laws are gradually dethroned by the organotypical, with the same result as that postulated by Minot.

In another respect, however, Hertwig differs from Minot. Minot maintains that cytoplasmic differentiation is irreversible, that having once started it must continue to senility and death. Hertwig, on the other hand, believes that under certain conditions the specialized cells can throw off the organotypical yoke and become again cytotypically active. His theory of cancer, for example, is that its cells are of this type, being successful revolutionists against the organotypical control.

There seems little doubt that Minot's position is untenable in this respect, except possibly for some of the most highly differentiated cells in the highest animals. The results of regeneration experiments in the lower animals (Child, 1913), and the recent successes in growing animal tissues in plasma (Carrel and Burroughs, 1910, Carrel, 1912, and others), seem to show conclusively that specialized tissue has not irretrievably lost its earlier powers.

For forty years the evidence from the plant side has been amply sufficient to show that Minot's views as to the irreversibility of differentiation would not hold for plant cells (Nemec, 1905). The formation of adventitious tissue, of secondary meristems, of budding and mature leaves, has shown the possibility of de-differentiation in specialized plant cells.

The writer's results attack Minot's theory in an even more vital point, since they indicate that senility occurs even in meristematic tissue and is not dependent on differentiation. Furthermore, Minot's own figures regarding the relative rapidity of the advance of senile degeneration, showing that its onset is most rapid at the very beginning, when differentiation is least, argues against the theory that specialization is the primary cause of senility.

Accumulation of katabolic products

Child (1912), in an extremely interesting paper, states his opinion as follows: " My experiments led me to the conclusion that senescence in its simplest terms consists in a decrease in the rate of metabolism determined by the gradual accumulation of relatively inactive structural obstacles to metabolism which in turn are the necessary consequence of continued metabolism under constant or relatively constant conditions and in the presence of nutritive material."

Hertwig, from his study of the " depression stages " in various protozoa, comes to the conclusion that senescence is due to the accumulation of materials in the nucleus until it becomes inert. He considers that a large amount of material must be eliminated from such an engorged nucleus before it can again become active. Child (1913), from his study of a planarian, which reproduces only asexually, and from a long series of regeneration experiments, has come to the conclusion that when such an accumulation occurs a condition of senility is reached, and rejuvenescence is restored only if the cell can rid itself of the accumulated inert substances. He is of the opinion, further, that hunger, removal of a part of the body, encystment, and asexual reproduction, are as efficient in this respect as sexual reproduction. He says, as regards the plants, there can be no doubt that the same is true.

Hertwig (1906) disagrees in part with this conclusion. He thinks that hunger, encystment, and special protozoan methods of nuclear destruction, are temporary patchwork repairs which, in forms capable of sexual reproduction, postpone senility but cannot produce the complete rejuvenescence that results from sexual reproduction. The evidence obtained from the studies on *Vitis vulpina* supports the view of Hertwig and disapproves Child's statement regarding certain plants at least. In forms that are without sexual reproduction and that are capable of continuous existence, the protoplasm must free itself from its accumulations more readily than protoplasm that depends on sexual reproduction.

It will be noticed that both Hertwig and Child picture the accumulation of inert products as interfering primarily with metabolism as a whole, not merely with katabolism. This accumulation theory must be discussed, as to its probability, in connection with the next theory of senility.

Decreased elimination of waste products

The first thorough treatment of the idea that senility may be due to the retention of poisonous waste in the tissues was that by Mühlmann (1900). His argument is based on the fact that as the growth of a many-celled organism goes on, the central cells are shut off from direct contact with oxygen, and become dependent for food, oxygen, and elimination of waste on the cells that still remain in contact with the surface. With increased growth, moreover, the mass increases as the cube, while the surface increases only as the square, of the dimensions. Therefore the number of dependent cells is increasing much more rapidly than the number of those cells that are able to excrete. The central cells are thus forced to retain their poisonous waste products longer and longer, and suffer in consequence an increasing degree of poisoning. Mühlmann makes the interesting observation that the organs which represent the surface continue to grow for the longest time — skin, lungs, intestines, vessels, and heart, which grow for from forty to fifty years after birth; while the musculature and the skeleton, which lie deeper in the body, stop their growth earlier, and the nervous system, which is deepest of all, grows but little after birth.

Montgomery (1906) gives an interesting argument along the same line. He says that death results from the insufficiency of the excretion process, and that this becomes impaired because the excreting surfaces do not keep pace with the increase in mass. He carries this idea so far as to postulate that spore formation and gamete production are essentially excretion processes, the spores or the gametes separating from the poisoned excrementitious soma. He exactly reverses the old Hildebrand and Weismann concept that the soma dies because the gametes have been produced and have gone, by claiming that the sex cells left because they could not endure the conditions any longer.

There are some very serious objections to be raised against the argument that harmful retention of waste products, due to the greater increase of mass than of surface, is the basic cause of senility. Considering first the condition in the human being, it is seen that the excretory surfaces continue to grow until the period of greatest activity is well past. Why should the excretory surfaces, which proved amply sufficient during the period of greatest activity, be unable to do their part after the organism as a whole is much less active?

Furthermore, if the amount of surface of the excretory organs is not sufficient to eliminate waste products fast enough for the internal mass of cells to continue to be active, how can be explained the rapid development of the unborn child, which must depend on its mother's excretory surfaces to eliminate not only her own tissues' waste but its own as well. It should be borne in mind that many of the human organs have a considerable excess power above that which is needed for ordinary excretions, and there are many evidences to show that the excretory organs can eliminate much more than they are usually called upon to do.

In this theory of the insufficiency of excretion, the "guilty-organ" theory is being met under a different form. It is not a single organ, but a single function, which is at fault this time.

When this theory of the insufficiency of excretion produced by the disproportionate increase in surface and mass is examined in the light of the facts reported for Vitis, important evidence against it is seen at once. In the perennial vine with its annually increasing number of leaves, there is no such discrepancy between increase in surface and mass as is found in animals, and yet here, as in animals, comes this same senile degeneration. If, therefore, there is a retention of harmful waste products in cells, it is not due to the apparently plausible disproportionate increase of volume and surface.

The retention of poisonous waste material, however, should be considered as important among the different possible causes of senility, as affecting the active cell. The immediate harm suffered by the human body from the lack of a pound of food is very much less than that which could be caused by the presence in the body of a minute quantity of poison. In other words, equal losses in efficiency in the functions of digestion and excretion may not be of equal harm to the cell. The loss in efficiency of excretion will affect the cell first. This is the argument which supports the idea that defective excretion may be the cause of senility, rather than the argument based on relative surface and mass.

But here again, if the efficiency of excretion becomes lessened, it must be due to some chemical or physical change occurring in the cell, and this change must then be the primary senile deterioration of which the decrease in excretion is a result.

A change of this nature seems to be indicated in the cells of the leaves of *Vitis vulpina* and certain other plants. A change in permeability, which would explain the decrease in size of the vein islets if present in all living cells, both plant and animal, would directly and inevitably decrease the efficiency of excretion of all cells both internal and external.

DECREASING PERMEABILITY AS AN EXPLANATION OF SOME OBSERVED SENILE DEGENERATIONS

It should be emphasized at this point that a decrease in permeability with age must be the result of some deeper-lying physical or chemical senile degeneration in protoplasm. It is probable that this fundamental cause produces other changes besides that of decreasing permeability. This will be touched upon shortly. With this in mind, it will be interesting to see to what degree a senile decrease in permeability will produce the effects which the other theories of senility assert are present and which they explain in their own way.

The presence of inert accumulations in cells would certainly be brought about by an increasing difficulty of exit from the cells. Furthermore, it is quite possible that decreasing permeability would favor the production of changed metabolic products. It is equally evident that a certain degree of impermeability is also essential to the cell, since on this depend the retention of food, the protection against harmful solutes in the environment, and the maintenance of turgidity. There must therefore be a constant compromise in the living cell between permeability and complete impermeability. It is possible that even in the youngest and most vigorous cell the degree of permeability required is such that oxygen cannot enter as rapidly as is needed at times of great activity. Under such partially anaërobic conditions, it is well known that certain cells, bacteria for example, may produce different katabolic products from those produced when abundant oxygen is available. Some of these products might be such as would merely interfere with cell activities by their accumulation, while others might be toxic in their action. It is quite possible that some of these changed products resulting from incomplete permeability might act upon the protoplasm in such a way as to further decrease its permeability. This would lead in turn to a more rapid production of the injurious substances. The presence of a decrease in permeability with age seems to account in part for the retention of inert accumulations, and possibly may be considered an important cause for the production of the accumulating material. It is interesting to note that this idea had occurred to Child (1912) in connection with senile degeneration in sperm cells, although he did not consider it as a general cause of senility.

The objections which may be raised against the theory that senility is due to the retention of toxic waste products owing to the lack of sufficient excretory surfaces in old organisms, do not apply when the retention of these substances is referred to a general decrease in permeability operating in all cells of the organism. A decrease in permeability as age increases accounts perfectly for every condition that the older theory claimed to exist. The loss of efficiency in excretion will occur in small organisms as certainly as in large ones, will characterize all cells and not simply a localized fraction, and does not require any postulates regarding relations between mass and surface, which are certainly untenable for the higher plants and probably for the higher animals. Furthermore, increasing permeability interferes with the inward movement of substances, and this undoubtedly is also an important consideration and perhaps in plants more important than difficulty of excretion.

Lillie (1909) argues strongly that many stimuli act on cells by the production of temporary increases in their permeability. It is also obvious that a complete cessation from activity tends to aid in the elimination of substances from the cells. Since the production of new metabolic products ceases, the cell is able to get rid of a certain amount of accumulated waste. Hunger, sleep, the division of the lower animals in regeneration experiments, encystment, and asexual reproduction, would therefore, in varying degrees according to method and organism and environment, utilize both these methods for decreasing the accumulations. The internal stimuli which are set in force in starvation effect katabolism of storage products that under normal conditions are unaffected, and in such cases the nature of the stimulus may also be such as to increase the permeability of the cells.

In the form in which no sexual reproduction occurs, it is possible that the accumulation of products resulting from the decrease in permeability, when it reaches a certain stage, produces a stimulus of such powerful nature that the original degree of permeability is restored. It is noteworthy, in this particular, that passive stages or encystment are common in such forms and aid in the process of waste elimination.

Is there any reason to believe that sexual reproduction rejuvenates through a restoration of the original degree of permeability? If senility is due in part to decreasing permeability, then rejuvenescence must be due in the same degree to increased permeability.

The effects of sexual reproduction can be most easily studied in the eggs of marine forms, which are normally fertilized when free in the water. A considerable number of researches on such material have been made by animal physiologists, and among the functions studied was this one of permeability. All these investigators have described a marked increase in permeability as the most characteristic effect of the entrance of the sperm into the ovum. This union of the two sex cells, therefore, generates a stimulus of such a powerful influence on permeability that the original degree of permeability is restored. Lillie (1909) has observed that pigments present begin to pass out rapidly; Lyon and Shackell (1910) found that dyes entered much more readily than before; while Loeb (1907), Warburg (1910), and others have observed that oxygen entered much more rapidly than before. Lillie (1909) thinks that this first remarkable increase in permeability lessens to a certain degree soon after; but this, if true, merely means that the first great increase in permeability is even greater than that characteristic of youth — one that is valuable for the sudden expulsion of accumulated waste, but too great to be suited for the retention of food, for turgidity, and for protection in the living embryo.

The evidence seems strong, therefore, both from the standpoint of senility and from that of rejuvenescence, that the duration of life is directly linked with the degree of permeability in that part of the living cell which places it in contact with the universe about it, and that as the activities of life proceed the cell is being gradually entombed by an inevitable decrease in the permeability of its protoplasm.

While decreasing permeability furnishes a possible explanation of the more obvious symptoms of senility, it cannot be the only degeneration of first rank. All protoplasmic functions must be involved. Underlying these primary causes of senile degeneration there must be some general fundamental cause from which they spring. This fundamental cause may well be the colloidal nature of protoplasm. While the physical attributes that characterize the colloidal condition of matter are not thoroughly understood, the crude generalization may perhaps be advanced that the unit of structure of the colloid is a molecular complex while that of the crystalloid is a single molecule. The physical changes which colloids undergo when exposed to external forces may be referred to rearrangements in the positions of the molecules in the unit complexes. The rise of the melting point of paraffin on repeated applications of heat, the reversible or irreversible changes from sol to gel under different degrees of heat, and other modifications of colloidal response produced by repeated applications of a given force, are examples of colloidal changes probably due to rearrangement of the molecules in the complex.

This intricate interrelation between the constituent parts of the colloidal unit of structure is subject to progressive changes in many non-living colloids, and these, with the passage of time, exhibit changes which might be called senile degenerations. The tendency to a closer approximation of the units of colloidal suspensions, as time goes on, is an illustration of the instability of the colloidal condition. Since the relatively simple relations in the nonliving colloidal complex tend to change on the application of external forces, or even with long standing, it seems inevitable that the extremely complex colloidal states constituting protoplasm should be progressively modified by the activities of life and the impact of external forces. The wonder is not that protoplasm is subject to senile change, but that the change is so slow.

If one of the changes in the colloidal units is a closer aggregation of the constituent parts, the probable effect on protoplasm will be to make it less permeable, lessen the quantity of imbibed water, and obstruct the other physical activities of life. Whatever the changes in the colloidal complex may be, they are the fundamental cause of senility.

Rejuvenescence is the process of restoring the original arrangement in the colloidal units of the protoplasmic colloids. Sexual reproduction is one method by which this is accomplished, while in sexless plants more primitive methods are used.

Whether the progress of senility in sexual plants and animals can be stopped, or even retarded, by such means of rejuvenescence as are utilized in sexless forms, and thus to a certain degree are applicable to somatic cells generally, is a problem for the future to solve. The present knowledge of the cause of senile degeneration does not justify an unqualified denial of the possibility of somatic rejuvenescence.

ACKNOWLEDGMENTS

The writer takes pleasure in expressing his indebtedness to Dr. Lewis Knudson, of Cornell University, and to Dr. B. M. Duggar, of the Missouri Botanical Garden, for many valuable suggestions in the preparation of this paper. He also acknowledges the leave of absence granted to him by his own institution, the University of Cincinnati, which enabled him to hasten the completion of this stage of the work.

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CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

A BACTERIAL DISEASE OF STONE FRUITS

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A BACTERIAL DISEASE OF STONE FRUITS

A BACTERIAL DISEASE OF STONE FRUITS[1]

F. M. Rolfs

HOST CONSIDERATIONS

Cultivated varieties of the apricot (*Prunus armeniaca*), the nectarine (*Prunus persica* var. *necturina*), the peach (*Prunus persica*), and the plum (*Prunus domestica*, *Prunus americana*, and others), are known to be affected by the bacterial disease discussed herein. It will be noted that the hosts are closely related.

THE DISEASE

NAMES

The disease on the foliage during its early development or in cases of slight infection is known as *bacterial leaf spot;* in more advanced stages or in more serious outbreaks it is termed *bacterial shot hole.* On the fruit it is sometimes referred to as *black spot*, the growers, however, more commonly speaking of it as *bacterial crack.* The disease on the young twigs has received the names *black spot* and *black tip*, and when open perennial wounds are formed on the branches these are called *bacterial cankers.*

HISTORICAL

The disease is apparently of American origin, it having been found, so far as is known, only in the United States. During the past nine or ten years fruit growers in various sections of the country have called attention to a shot-hole disease of peach and plum foliage, which in wet seasons has in many cases caused premature defoliation. O'Gara (Rorer, 1909)[2] reports a shot-hole condition of peach foliage prevalent in Georgia, and suggests that it is caused by a bacterial organism. Smith (1903) calls attention to a bacterial disease of Japanese varieties of plum in central Michigan, which appears as a shot hole of the leaves and a black spot of the fruit. Clinton (1904) describes a leaf spot on peach in Connecticut, which he attributes to a bacterial organism. Heald (1906) discusses a disease, apparently of the same nature, found in Nebraska on the twigs

---

[1] Also presented to the Faculty of the Graduate School of Cornell University, June, 1913, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

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[2] Dates refer to literature cited, page 435.

and branches of Wickson and Whitaker varieties of plum. Jackson (1907) reports a similar disease from Delaware, and states that considerable injury results to the plum orchards in that State. Smith (1905 b, figs. 11, 12, 15, 70, 72, pl. 19) gives diagrams of sections through diseased plum leaves and fruit tissue, and illustrations of diseased plum fruit. Rorer (1909) records a detailed study of the disease on leaves, twigs, and fruits of peach. Smith (1911) discusses the disease on plum leaves and fruits, and gives illustrations of diseased plum fruits as well as sections through diseased fruit tissue. Heald and Wolf (1912) report the occurrence of a bacterial canker on plum at San Antonio, Texas. Lewis (1912) describes a bacterial canker on plum twigs and records the results of several inoculation experiments. He gives illustrations of cankers on twigs four and six years of age.

The disease was first brought to the attention of the writer in 1904, when he was Botanist and Horticulturist at the University of Florida. Many letters were received from fruit growers in northern Florida and southern Georgia, complaining of a shot-hole condition of peach and plum leaves accompanied by considerable premature defoliation. The foliage of the different varieties of peach and plum trees in the university horticultural orchard also developed the shot-hole condition, and in most cases fell prematurely. Other duties prevented the writer from undertaking extensive investigation of the problem at that time. However, on taking up work at the Missouri State Fruit Experiment Station in 1906, he found that the blackened areas, so common on the peach twigs in northern Florida in 1905, were even more abundant on the peach and plum twigs in Missouri. Additional observations have brought out the fact that the disease is more or less injurious also to the apricot and the nectarine.

GEOGRAPHICAL DISTRIBUTION

It is not easy to obtain information as to the distribution of this disease, since a bibliography of the literature relating to shot-hole injury of stone fruits shows that the injury is usually attributed to the work of fungi. This is not surprising, as lesions resulting from various causes resemble one another so closely that it is often impossible to determine exactly the causal factor without the aid of a microscope, and it is not always possible even with a microscope. The disease has been reported by investigators as occurring in Arkansas, Connecticut, Delaware, Georgia, Michigan, Nebraska, and Texas. Diseased material has been obtained by the writer from Alabama, Arkansas, Florida, Georgia, Illinois, Iowa, Kansas, Michigan, Missouri, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, and Texas.

ECONOMIC IMPORTANCE

Bacterial leaf spot, or shot-hole as it is usually called, is one of the chief foliage diseases of stone fruits in the humid districts of the South. It occasionally becomes more or less aggressive also in some sections of the northern and eastern States. Its importance varies from year to year, there being an abundance in wet seasons and little in dry seasons. The disease appears to be more severe in warm, humid regions than in cool, dry sections. In southern Missouri its economic importance has kept pace with the growth of the peach industry, and it is now one of the most difficult diseases with which the fruit grower has to contend. Its attacks on the plum have made it practically impossible to grow certain varieties of Japanese plums on a commercial scale. The fruit of some varieties of apricot and nectarine also are injured more or less by the disease, but since apricots and nectarines are not extensively grown in Missouri the disease on these fruits has not attracted much attention.

In different parts of Missouri, during 1908 and 1909, a record was made of the amount of infected fruit in diseased Elberta peach orchards, in order to determine the extent of the injury due to this disease. It was found that from 1 to 10 per cent of the fruit was infected in orchards well cared for, and from 25 to 75 per cent in orchards poorly cared for. The lesions on the fruit are in many cases not sufficiently prominent to mar its appearance greatly, but the larger proportion of such fruit is graded as "seconds." None of the fruit holds up well in shipping, and in some cases it is completely destroyed by various rot-producing organisms before being placed on the market.

It is especially difficult to obtain reliable figures on the loss due to this disease, since the loss is not confined to the direct effect on the fruit but must include also the influence of the diseased foliage on the general vigor of the tree. Severe outbreaks soon impair the efficiency of the foliage, and if they occur early in the season they reduce the size and greatly lower the quality of the fruit. Severe foliage injury also has a marked influence on the formation of fruit buds, and trees that have their leaves injured early in the season usually carry a very light crop of fruit the following year. Young trees are usually more affected than those six or seven years old, and badly diseased young trees soon become stunted and in some cases permanently injured.

SYMPTOMS

The disease affects the leaves, twigs, and fruit in a characteristic manner. It develops on twigs and shoots of only the current year's growth, but the wounds on the twigs often persist as open perennial cankers.

The very common fungous disease of apricot and plum foliage due to *Cylindrosporium padi* is similar in its symptoms to bacterial leaf spot, but usually can be distinguished from the latter by the white downy growth on the lower surface of the spot. Pierce (1894) reports a similar disease on almond foliage in California due to the work of *Cercospora circumscissa*. There is also a leaf spot of unknown cause which is constantly associated with peach and plum rosette. Spray mixtures and other toxic substances applied to the leaves often produce an injury which is difficult, without the aid of a microscope, to distinguish from bacterial leaf spot. Care must be taken not to confuse the lesions due to these various agents.

On the leaves

The first evidence of the disease on the leaves is the appearance of small gray specks (Fig. 59), which become somewhat angular in form and soon take on a water-soaked appearance. They later become brown or purplish brown, in some cases scarlet, and finally change to dark brown. The spots measure from one to five millimeters in diameter and when numerous they coalesce, so that large areas of the leaf may be involved. A more or less circular diseased area is finally separated by a line of cleavage from the healthy tissue, the injured tissue within this area promptly contracting, drying, and falling out. This gives the leaf the shot-hole appearance. On the plum, in some cases, after the disease has apparently run its course and the spot is well formed, a second and even a third period of invasion may follow and an area one-half inch or more in diameter may develop from a single spot. Leaves that are badly diseased soon fall to the ground. It is not unusual for an infected tree to lose from fifteen to twenty-five per cent of its leaves by August, and in the more extreme cases as high as seventy-five per cent, or even more, of the leaves

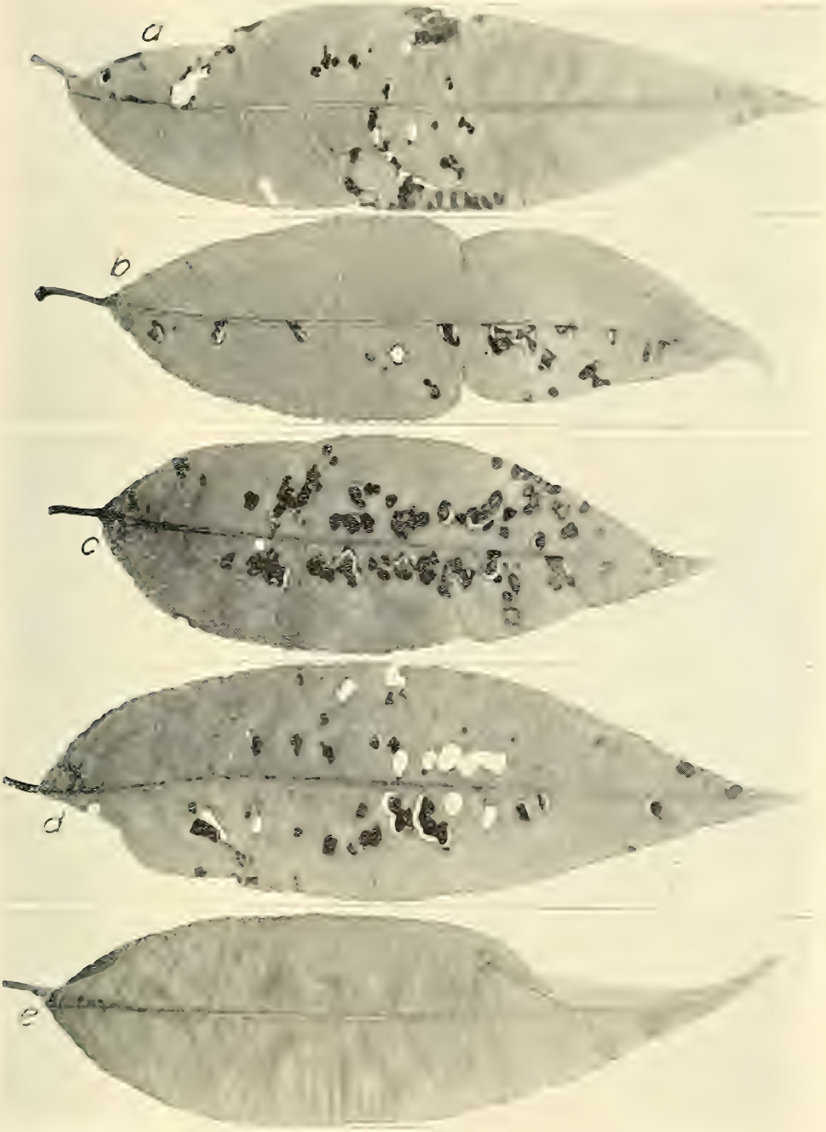

Fig. 59.—*Elberta peach leaves inoculated with pure cultures of Bacterium Pruni. The growth from a seven-days-old-slant Hiss-glucose culture was thoroughly mixed in one hundred cubic centimeters of sterile water and applied to the leaves by dipping the fingers into the water and then rubbing the leaf between the thumb and the fingers. Inoculations were made on July 16, 1912, and the photograph was taken on August 28, 1912*

a, *Inoculated with a pure culture obtained from an apricot twig*
b, *Inoculated with a pure culture obtained from a nectarine twig*
c, *Inoculated with a pure culture obtained from a peach twig*
d, *Inoculated with a pure culture obtained from a plum twig*
e, *Check. Sterile water was applied to the leaf*

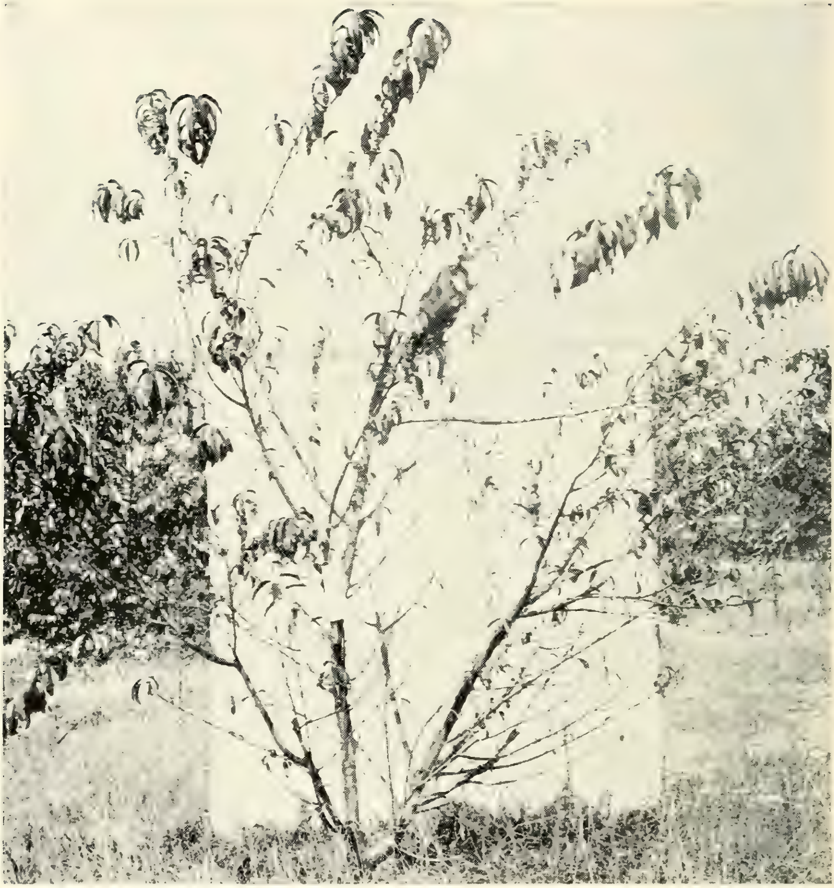

Fig. 60.—*Arp Beauty peach tree showing defoliation due to the work of Bacterium Pruni. Most of the leaves that formed before July 1, 1909, have fallen. All leaves still on the tree are more or less injured.* (*Photograph made on September 6, 1909*)

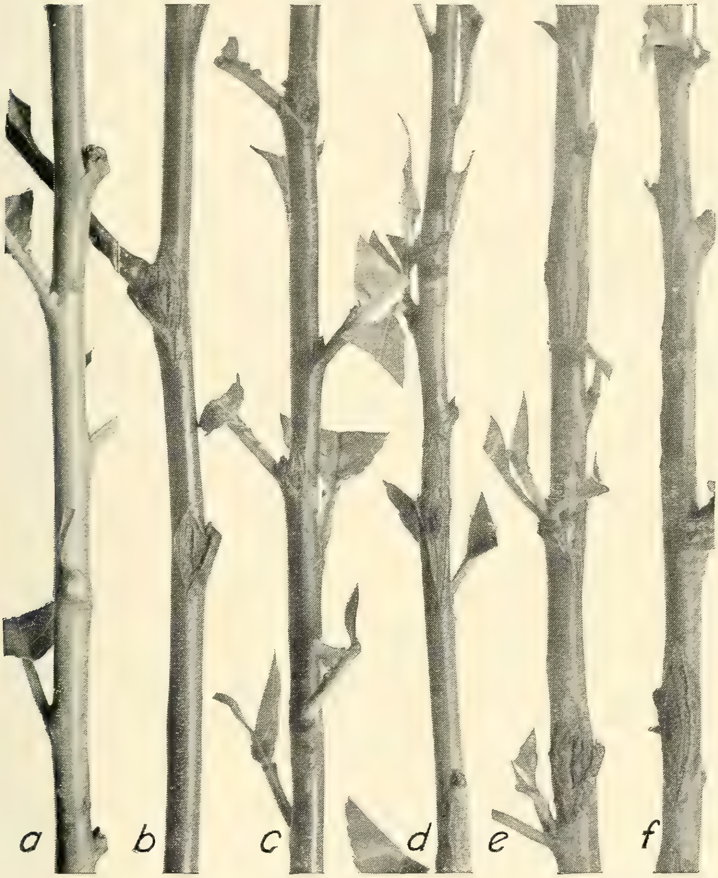

FIG. 61.—*Elberta peach twigs inoculated with pure cultures of Bacterium Pruni. The growth from an eight-days-old-slant Hiss-glucose culture was thoroughly mixed in one hundred cubic centimeters of sterile water. Each twig was inoculated at four points by inserting the point of a hypodermic needle just below the cuticle and forcing the water containing the organism into the tissues. Inoculations were made on June 25, 1912, and the photograph was taken on August 20, 1912*

a. Check. Sterile water was used
b and c, Inoculated with a pure culture obtained from an apricot twig
d, Inoculated with a pure culture obtained from a nectarine twig
e, Inoculated with a pure culture obtained from a peach twig
f, Inoculated with a pure culture obtained from a plum twig

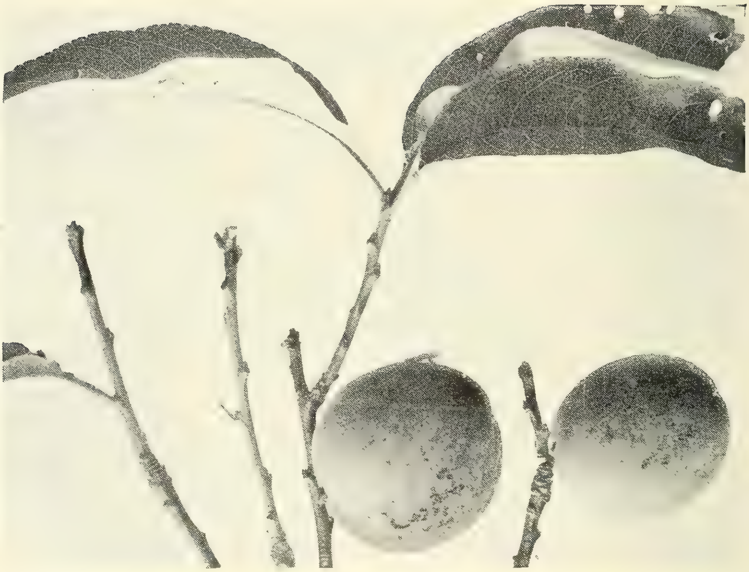

FIG. 62.—*Elberta peach twigs, leaves, and fruit, showing injury by Bacterium Pruni. The black tips of the twigs are to be noted particularly.* (*Photograph made on August 15, 1909*)

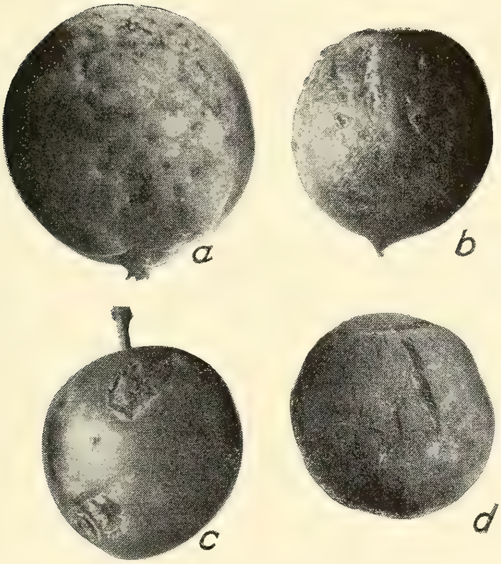

FIG. 63.—*Naturally infected fruits of peach, nectarine, plum, and apricot: a, Crothier peach; b, Victoria nectarine; c, Abundance plum; d, Bread apricot. (Photograph made on July 1, 1909)*

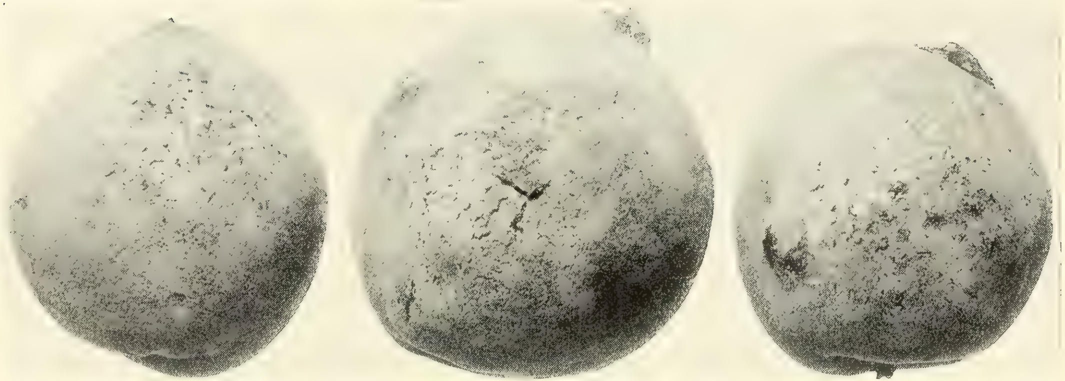

FIG. 64.—*Elberta peach fruit showing injury by Bacterium Pruni. The tree was sprayed seven times. The first application was made with lime-sulfur solution, 15-15-50 formula, on* **March** *12, shortly before the fruit buds had opened. The other six applications were made with self-boiled lime-sulfur mixture, 12-8-50 formula. The first of these applications was made on* **April** *21, just after the leaf buds opened; the others followed at intervals of about twelve days. Fifty-two per cent of the fruit was dotted* **with numerous cracks.** *(***Photograph made** *on* **August** *12, 1908)*

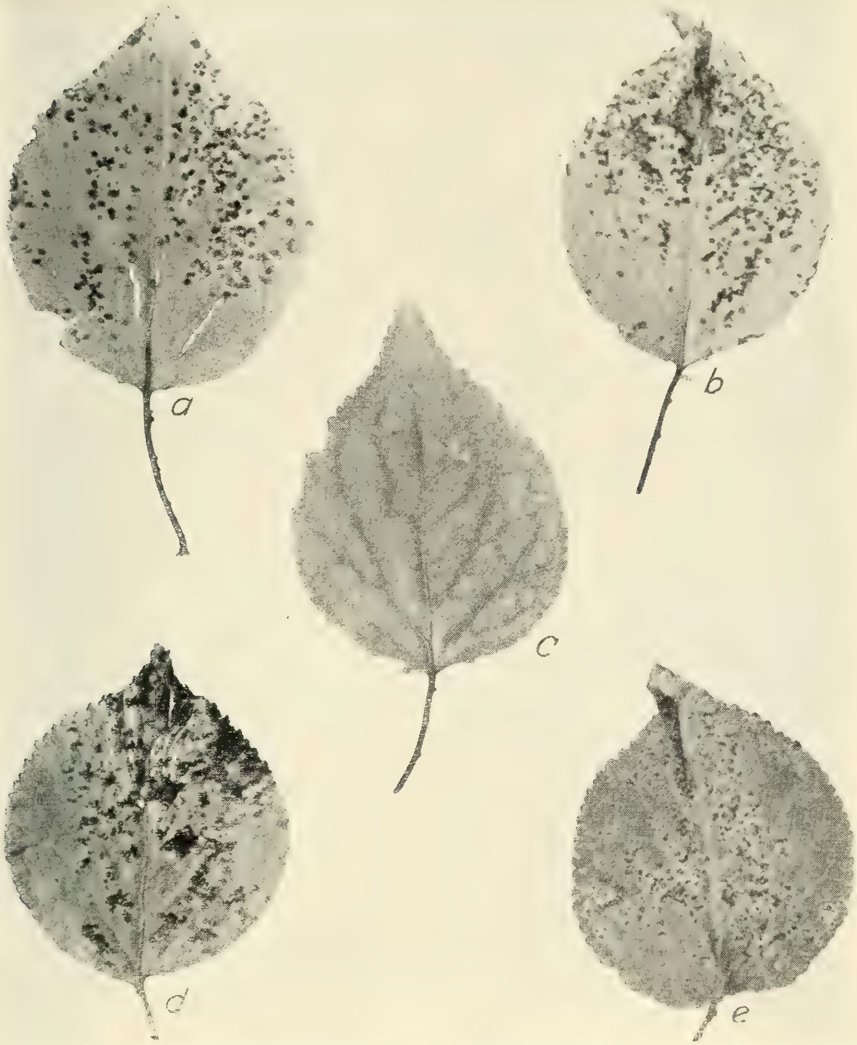

FIG. 65.—*Alexander apricot leaves inoculated with pure cultures of Bacterium Pruni. The growth from a six-days-old-slant Hiss-glucose culture was thoroughly mixed in one hundred cubic centimeters of sterile water and then applied to the leaves by dipping the fingers into the water and gently rubbing the leaves between thumb and fingers. Inoculations were made on June 29, 1912, and the photograph was made on August 27, 1912*

a, Inoculated with a pure culture obtained from an apricot twig
b, Inoculated with a pure culture obtained from a nectarine twig
c, Check. Sterile water was applied to the leaves
d, Inoculated with a pure culture obtained from a peach twig
e, Inoculated with a pure culture obtained from a plum twig

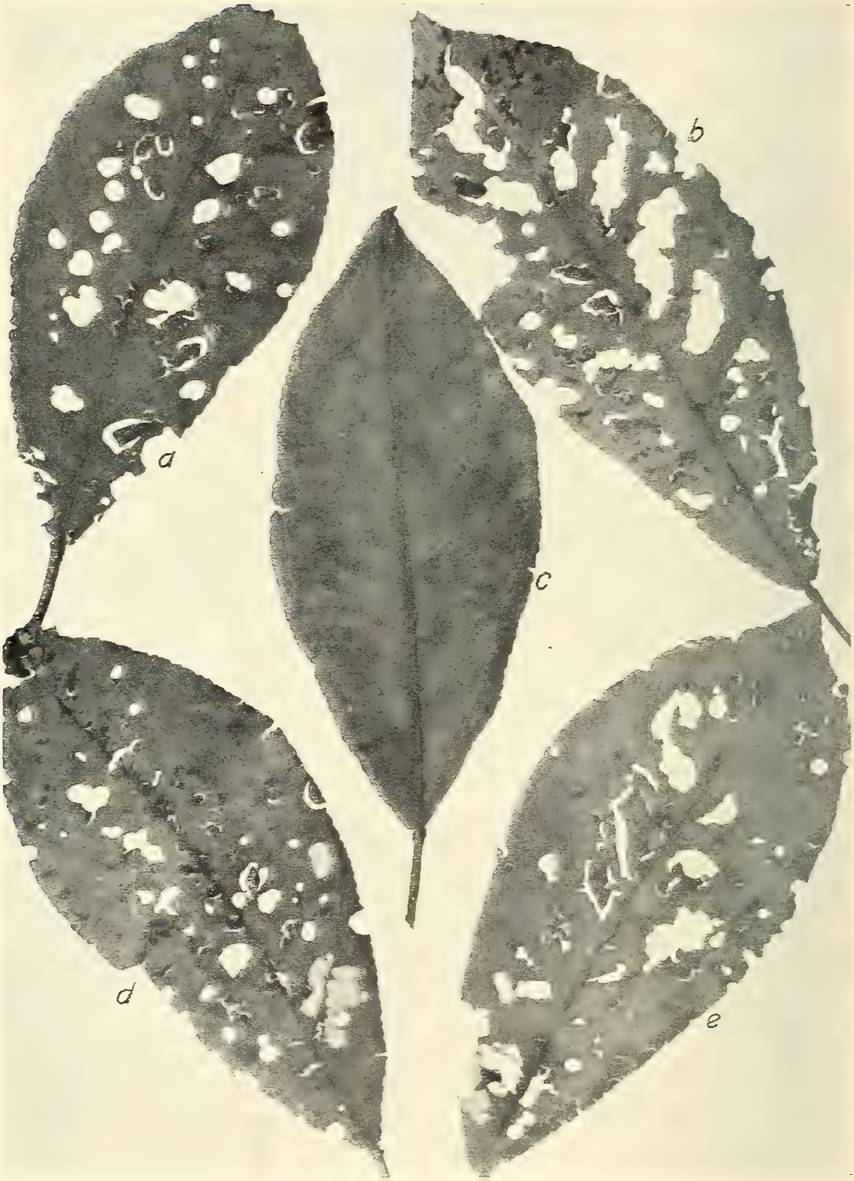

FIG. 66.— *Burbank plum leaves inoculated with pure cultures of Bacterium Pruni. The growth from a seven-days-old-slant Hiss-glucose culture was thoroughly mixed in one hundred cubic centimeters of sterile water and applied to the leaves by dipping the fingers into the water and then gently rubbing the leaves between thumb and fingers. Inoculations were made on June 27, 1912, and the photograph was made on August 27, 1912*

a, Inoculated with a pure culture obtained from an apricot twig
b, Inoculated with a pure culture obtained from a nectarine twig
c, Check. Sterile water was applied to the leaves
d, Inoculated with a pure culture obtained from a peach twig
e, Inoculated with a pure culture obtained from a plum twig

fall. The older, first infected leaves are usually, although not always, the first to go. One leaf after another falls, leaving finally only a tuft of young leaves at the tip of each twig and giving the tree a characteristic appearance (Fig. 60).

On the twigs

In some seasons black cankers form on the twigs in early spring, but as a rule these do not appear in abundance until May or June. The first visible indication of the canker is the appearance on the young shoots of a water-soaked spot surrounding a lenticel. In some cases the diseased area bulges out, more or less, in the beginning. As the spot enlarges it soon elongates. Single spots measure from one to four centimeters in length and often extend from one-half to two-thirds of the way around the shoot. The area becomes more or less sunken in age, soon becomes brown or purplish brown, then dark brown, and finally purplish black or black (Fig. 61). It frequently happens, especially on the nectarine and the peach, that several of the spots become confluent, forming areas which extend from two to three inches along the stem and in some cases completely girdle it. In favorable seasons the tips of many of the shoots are entirely blackened and killed (Fig. 62), this condition being most prominent on the nectarine and the peach. In most cases the injury is overcome gradually, and as a rule all traces of these black cankerous spots disappear in the second year.

The black spots on apricot twigs also disappear for the most part, but on plum twigs many of them persist from year to year, forming perennial cankers. As many as twenty-two cankers in different stages of development have been counted on a single plum twig (a water sprout). Open cankers also form on the apricot, the nectarine, and the peach, but they are far less abundant on these trees than on the plum.

The disease is usually more aggressive during the first two or three years after planting than it is later. It is not only likely to defoliate the young trees prematurely, but in many instances it kills back the twigs and frequently stunts the trees. A case of this sort was under observation, in which two hundred Elberta peach trees from an infected nursery were mixed with a shipment of twenty-three thousand healthy trees. Within three years from the time of planting, every tree in the orchard was more or less diseased, and many of the trees remained small and produced small, inferior fruit.

On the fruit

The disease does not ordinarily appear on the fruit until about the middle of May. Injured fruits of all the four kinds of hosts, however, have been collected early in May (Fig. 63). The first external signs of the disease are numerous small gray specks just below the cuticle. These specks enlarge slightly and soon take on a water-soaked appearance or a purplish color. The skin over the spots is soon ruptured, producing numerous straight or angular cracks which resemble the wounds on chapped hands (Fig. 64). Usually individual spots do not measure much more than two millimeters in diameter, but they are often so numerous that the cracks soon become confluent and frequently form a network, fissures nearly an inch in length being developed in some cases. On young growing fruit it is not uncommon to find rifts extending nearly the entire length of the fruit. Such wounds mar its appearance and in many cases render it worthless for market.

On all kinds of fruit the disease results in the formation of these small, more or less abundant, crack-like wounds, but certain varieties of the nectarine are especially liable to this type of injury. In some cases the spots gradually widen and extend for a considerable depth into the tissue, forming roundish, sunken, black spots which finally measure from one-fourth to one-half inch in diameter. Rifts develop in the blackened tissue. These widen, and cracks appear throughout the diseased area which often (especially when the disease is on young developing fruit) finally extend beyond the dead into the healthy tissue. In many cases the fruit becomes so badly distorted and cracked that it is worthless, and it is soon destroyed by various rot-producing organisms.

ETIOLOGY

Historical

The pathogen causing this disease was first isolated by the writer in 1904 at the University of Florida. Many poured plate cultures were made from the spots on injured leaves of both peach and plum. Cultures made from the young water-soaked spots invariably gave numerous yellow bacterial colonies, and in no case was a species of Cercospora or Cylindrosporium obtained. Consequently it was concluded that the disease was of bacterial origin. It was observed also that the defoliated twigs sometimes showed one or more blackened, slightly sunken areas. These often measured as much as seven millimeters in width and extended from five to fifty millimeters along the stem, in some cases the entire end of the twig being blackened. Cultures from these areas produced yellow bacterial colonies.

On taking up work at the Missouri State Fruit Experiment Station in 1906, the writer observed that the twigs of some varieties of peach and plum in Missouri also showed blackened, sunken areas, and poured plate cultures from the diseased tissue in many cases gave yellow bacterial colonies. About the middle of June, 1906, it was observed that the foliage of trees bearing the diseased twigs had developed the shot-hole condition to a marked extent, while the foliage on trees with twigs free from the blackened areas was quite free from shot-hole. The fruit on Abundance and Burbank plum soon developed many typical black-spot areas, from which pure cultures of a yellow organism were easily obtained. A little later many peach fruits developed numerous small cracks. These were at first supposed to be due to the work of curculio, but a careful examination showed bacteria to be present in enormous numbers.

A careful study soon proved that the organism had invaded the apricot and the nectarine in much the same way as it had the peach and the plum. The fruit of the nectarine in the station variety orchard in 1906 was so badly cracked as a result of the attack of this organism that the entire crop was destroyed by various decay organisms long before the ripening period. Some of the apricot fruits developed the typical black spot. The twig cankers on both apricot and nectarine were fully as plentiful as on the peach, and premature defoliation also occurred on both apricot and nectarine trees.

Nomenclature

Since bacteriologists have not yet adopted an international code of nomenclature, it does not seem advisable to enter here into an extended discussion of the limits of genera, the selection of types, or the position of this organism in the various systems of classification proposed. The writer has therefore elected to follow arbitrarily the suggestion given by Erwin F. Smith (1905a). The organism was first described by Smith (1903) under the name *Pseudomonas Pruni*. Later, however, it became *Bacterium Pruni*, when Smith substituted the generic name Bacterium Cohn emend. for Pseudomonas Migula.

Pathogenicity

Conclusive proof of the pathogenic nature of this organism was obtained in 1904 by Smith (1905 a), by inoculating the foliage and the fruit of Abundance plum. The pure culture of the organism used in inoculation was obtained from plum fruit of the same variety. The organism from several young-slant agar cultures was scraped into two hundred cubic centimeters of sterile water and sprayed with an atomizer on the foliage and fruit of a five-years-old tree. Diseased spots developed on both leaves and fruit in from ten to twelve days and became numerous after fourteen days. Poured plates from the fruit and the leaves of the inoculated trees yielded numerous colonies of the yellow organism. In 1907 Smith (1908) also successfully inoculated the foliage of a peach tree with the plum organism. Rorer (1909) isolated the organism from peach leaves in 1906, and the following year made a series of successful inoculations with it on the foliage of Elberta peach trees at Bentonville, Arkansas. His results agree in every way with those obtained by Smith with the plum organism. Rorer also obtained pure cultures of the organism from peach twigs and reported its occurrence on peach fruit. He found the cultural characters of the plum and peach organisms to be exactly the same.

In 1907, at Mountain Grove, Missouri, the writer repeated and confirmed Smith's experiments on the plum, and extended the infection experiments to apricot, nectarine, and peach. The organism used was isolated from an Abundance plum fruit and grown in pure culture. The organism was scraped from several slant agar cultures into one hundred cubic centimeters of sterile water, and sprayed with an atomizer on the fruit and leaves of eight-years-old trees of Royal apricot, Victoria nectarine, Elberta peach, and Abundance plum. The inoculations were made during a light, drizzling rain. Disease spots developed on fruit and leaves of all four hosts within twelve days, and after fifteen days they became very numerous. Poured plates from the fruit and the leaves of the inoculated trees yielded numerous colonies of the yellow organism. However, several of the check leaves on the apricot and the nectarine trees also developed spots from which the yellow organism was obtained. Pure cultures of the organism were obtained by the writer from leaves and twigs of apricot, nectarine, peach, and plum. These strains and the one obtained from an Abundance plum fruit were grown side by side on various culture media, and were exactly alike in all cultural characters.

In 1909, at Mountain Grove, cross inoculations with the four strains obtained from the leaves were made on eight-years-old Royal apricot, Victoria nectarine, Elberta peach, and Abundance plum trees. Two hundred leaves, fifty fruits, and twenty twigs were used in each case. The organism from young-slant Hiss-glucose cultures was thoroughly mixed with sterile water and sprayed on the leaves and the fruit with an atomizer. Each of the twenty twigs was inoculated at four points with a hypodermic syringe. Spots appeared on the leaves of all the inoculated trees in ten days, and on the fruit in from twelve to fifteen days. The inoculated tissue on the twigs assumed a water-soaked appearance in from four to eight days, becoming dark brown and finally, in about twenty-five days, almost black. Poured plate cultures from the spots on leaves, fruit, and twigs yielded numerous colonies of the yellow organism. Three of the check leaves on peach also developed a number of brown spots.

In 1910, at Mountain Grove, cross inoculations with strains of the organism from the fruit of the four hosts were made on eight-years-old Royal apricot, Victoria nectarine, Elberta peach, and Burbank plum trees. Fifty leaves and ten twigs were used in each case. The organism from young-slant Hiss-glucose cultures was scraped into sterile water. The fingers were dipped into this water, and the material was applied to the lower surface of the leaf by gently rubbing the leaf between thumb and fingers. Each twig was inoculated at six points with a hypodermic syringe. Gray spots appeared on the leaves of all trees in from six to seven days, and water-soaked spots appeared in great numbers about the tenth day. The inoculated tissue on the twig assumed a water-soaked appearance in from five to seven days and became dark brown in about twenty-five days. Poured plates from the spots on leaves and twigs yielded colonies of the organism more or less abundantly. All the twig check wounds soon healed, and the check leaves remained free from spots.

In 1911, at Ithaca, New York, cross inoculations with the four strains obtained from the twigs of the four different hosts were made on three-years-old Elberta peach and four-years-old Abundance plum trees. The organism from young-slant Hiss-glucose cultures was thoroughly mixed with sterile water and sprayed on the leaves with an atomizer. Twelve twigs were used in each case, and each twig was inoculated at five points with a hypodermic syringe. Spots on the leaves developed in nine days, and after twelve days water-soaked spots appeared abundantly. The inoculated tissue on the twigs assumed a water-soaked appearance in most cases by the seventh day. The typical black-spot and black-tip condition of the twig did not develop until after the twenty-fifth day. Poured plate cultures from the young leaf spots and the black spots on the twigs yielded colonies of the organism abundantly. Pure cultures of the organism from the twigs of the four kinds of trees were grown side by side on various culture media for six weeks, and in all cultural characters the strains were the same. All check wounds on the twigs soon healed, and the check leaves remained free from spots.

In 1912, at Ithaca, cross inoculations with strains of the organism from twigs of the four hosts were made on three-years-old Alexander apricot, Early Violet nectarine, Elberta peach, and Burbank plum trees (Figs. 59, 65, and 66). One hundred leaves and from twelve to twenty twigs were used in each case. The organism from young-slant Hiss-glucose cultures was scraped into sterile water. The fingers were then dipped into the water, and the material was applied to the lower surface of the leaf by gently rubbing the leaf between thumb and fingers. Each twig was inoculated at from three to five points with a hypodermic syringe (Fig. 61). Spots on the leaves appeared in from five to seven days, and water-soaked spots became numerous after the eighth day. The inoculated tissue on the twigs took on a water-soaked appearance in about seven days, but did not become dark brown until after the twenty-fifth day. Both leaf and twig spots yielded the characteristic yellow colonies on poured plate cultures. All check wounds on the twigs soon healed, and the check leaves remained free from spots.

Morphology

Vegetative cells.— The organism is a rather short rod, with rounded ends. It is found singly, in pairs, and, in liquid media, often in short chains. Individuals from a forty-eight-hours-slant Hiss-glucose culture are from 0.8 to 0.9 μ in length and from 0.3 to 0.5 μ in width. Bacteria taken directly from the twig cankers and stained with gentian violet measure from 1.6 to 1.8 μ in length and from 0.4 to 0.6 μ in width.

Endospores.— No endospores have been observed.

Flagella.— The organism is motile by means of one or several polar flagella. Both Pitfield's and Moore's flagella stains were used with good results. The flagella were also stained without a mordant, by exposing the covers to carbol fuchsin for from twenty-five to fifty minutes and carefully washing in alcohol (Fig. 67).

Capsules.— Capsules have frequently been stained in material obtained from Uschinsky's solution by both Welch's and Moore's methods.

Zooglœæ.— Zooglœæ are frequently found on various media.

Involution forms.— No involution forms have been observed.

Staining reactions.—The organism stains readily with the ordinary aqueous stains — aniline gentian violet, and carbol fuchsin.

Gram's stain.— Many efforts at staining were made with Gram's method, but the results were not sufficiently sharp and decisive to warrant a conclusion. In no case was the stain completely lost, nor was it ever retained in the original intensity. The aniline gentian violet and iodine solutions were used for two minutes and the preparations were left in absolute alcohol for three minutes. In no case were the mounts washed in alcohol as intensely stained as those not treated with alcohol, and consequently the organism must be regarded as Gram negative.

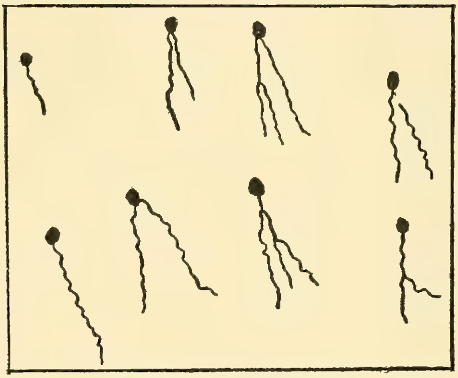

FIG. 67.—*Flagella of Bacterium Pruni. Stained from a culture of the peach strain of the organism grown in bouillon*

Acid-fast.— The organism is not acid-fast.

Cultural characters

Source of cultures.— Eighteen strains were used for each test, one from the apricot, one from the nectarine, nine from the peach, and seven from the plum. The reactions of all the strains on the various media were uniform, and no marked differences were observed except in one of the plum strains, which invariably grew most rapidly. The growth of this strain on potato plugs was more abundant than that of any of the other strains. Its growth on Hiss-glucose was often a number of hours in advance of the other cultures. Where there was any difference in the reaction one of the normal plum strains was always used as a basis for comparison.

Nutrient agar colonies.— When plantings were made from bouillon cultures six days old at room temperature of about 25° C., the colonies appeared as mere specks in from forty to forty-eight hours. Plantings made from diseased host tissue developed colonies in from three to ten days (usually from four to seven). No growth occurred at 37° C. The colonies were moist and glistening, and at first were almost white but later became amber yellow.[3] When not crowded, the surface colonies were round and reticulate, and reached a diameter of about one to five millimeters in ten days, with a smooth surface, an entire edge, and a finely granular internal structure. The deeper colonies were elliptical or lenticular. On plates twenty-five days old, colonies measured from ten to twenty-five millimeters in diameter. They had dark, translucent centers surrounded by a much lighter, often concentrically ringed, zone.

Nutrient agar streaks.— On slanted agar the streak made with a platinum needle developed a moderate amber-white (4) growth. The streak widened slowly, chiefly toward the bottom, made a filiform growth, and was raised, with a slightly contoured surface and an entire edge; it was translucent, with a glistening luster, and was slightly viscid; it did not color the agar on which it grew, at least not for a number of weeks. Growth was moderate. After five or six days the growth began to flow downward, and finally accumulated in an amber yellow (1), slimy mass at the base of the slant.

Nutrient agar stab.— On agar stab the growth was filiform, and was best toward the top of the stab, there being very little or no growth in the lower half of the medium. A circular colony formed on the surface at the point of inoculation, and gradually spread, completely covering the surface in about seven or eight days with a straw yellow (2) growth. The agar was not liquefied nor softened.

Gelatin colonies.— On gelatin plates at room temperature of about 25° C., the colonies appeared as mere specks in from forty-five to fifty hours. They were at first almost white, translucent, moist, and glistening. On the fourth day they measured about one millimeter in diameter. Both buried and surface colonies were round, finely granular, and with an entire edge, becoming with age amber yellow (2) in color. Liquefaction began usually on the third day, the colonies becoming saucer-shaped, spreading, and liquefying the medium in twelve days or more.

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[3] All the color terms used in this paper are based on René Oberthür's *Répertoire de Couleurs*, published by La Société Française des Chrysanthémistes in 1905.

Gelatin streaks.— On slanted gelatin (+15) at room temperature of about 25° C., the streak having been made with a one-millimeter loop, a light, semi-transparent growth was noticed after two days along the entire path of the loop. In four days there was a slight liquefaction along the streak. On the fifth day the growth was amber yellow (1) in color, liquefaction had extended the entire length of the streak, and the growth was about ten millimeters wide and five deep. On the eighth day all material in the slant down to three millimeters below the base of the slant had been liquefied. Complete liquefaction occurred in from twelve to thirty days.

Gelatin stab. — In gelatin stab the path of the needle became beaded, but usually in the upper twenty-five millimeters of the medium it at first became more or less filiform. The growth on the surface was most rapid, and liquefaction, which began about the third day, soon passed to the walls of the tubes and then slowly downward. The growth in the depth of the gelatin soon ceased and the material along the track of the needle was not liquefied more rapidly than that away from its path. The liquefied gelatin was at first more or less clouded and was of about the same color as the solid material, but later it cleared and took on a raw sienna (2) color. A bacterial layer Naples yellow (3) in color soon formed on the solid gelatin and gradually settled as the material liquefied. Both a rim and a pellicle, cream yellow (2) in color, finally formed. Complete liquefaction occurred in from several days to six weeks or longer, depending on the temperature, the strain of the organism, and the composition of the culture material. In four months the liquefied material was viscid, was raw sienna (4) in color, and had a thick, slimy, Naples yellow (3), bacterial deposit in the bottom of the tube.

Hiss-glucose colonies.— Plantings made on Hiss-glucose from six-days-old bouillon cultures came up in from two to ten days (usually from four to seven) at room temperature of from 23° to 25° C., forming colonies from one to four millimeters in diameter in about eight days. No growth occurred at 37° C., but at 25° C. colonies appeared in forty-eight hours. The colonies were at first almost white, translucent, moist, and glistening, but later became amber yellow (1). When not crowded, the surface colonies were round, with an entire edge, the surface being smooth with a more or less convex elevation, and the internal structure being finely granular. The deeper colonies were elliptical or lenticular in form. Plates containing twenty cubic centimeters of the medium developed single surface colonies in thirty days which measured from sixteen to thirty millimeters in diameter. The older colonies were amber yellow (4) in color, were convex, showed a dark central nucleus, and were often concentrically ringed. The smaller, thin colonies had a semi-transparent luster.

Hiss-glucose streaks.— On Hiss-glucose slant-tube medium (+15), the streak having been made with a millimeter loop, growth was noticed in forty-eight hours at room temperature of about 25° C. A convex, slimy, straw yellow (2) growth developed along the path of the loop. The streak widened gradually (chiefly toward the bottom), soon became filiform, and in ten days completely covered the slant with a slimy yellow (2) growth. In culture three or four months old the medium was browned. Some of the growth soon moved downward and formed a slimy deposit at the base of the slant, which finally became amber yellow (4) in color. As the medium dried out the slimy deposit gradually settled, and in the five- and six-months-old cultures there was a slimy, amber yellow (4) bacterial deposit from five to ten millimeters deep in the bottom of the tube. Inoculated tubes placed in the incubator at about 37° C. also covered the slants in ten days, but the growth was of about the same color as the medium.

Hiss-glucose stab.— In Hiss-glucose stab the growth was filiform and best toward the top of the stab, there being very little or no growth in the lower part of the tube. A circular growth formed on the surface at the point of inoculation, and covered the surface in six or seven days. The medium was not liquefied nor softened.

Potato agar streaks.— In potato agar a slightly raised, wet, shining growth developed along the path of the needle in two or three days at room temperature of 25° C. The streak gradually widened, and finally covered about two-thirds of the surface of the slant, becoming amber yellow (1) in color. Some of the slimy growth soon moved downward and formed a deposit at the base of the slant. Growth was moderate, never completely covering the slant.

Dextrose litmus agar streak.— In dextrose litmus agar, there was slight growth along the path of the needle in two days. The streak was slightly elevated, and widened most rapidly at the bottom, becoming filiform. The slimy growth passed downward and accumulated at the base of the slant, and the medium below the mass became entirely bleached in five or six weeks.

Lactose litmus agar streak.— Growth in lactose litmus was not so marked as on the dextrose litmus medium. There was slight growth along the path of the needle by the close of the second day. The streak was slightly elevated, and it gradually widened and became filiform. The slimy growth passed downward and accumulated at the base of the slant. Growth was less abundant, and bleaching below the slimy mass only slight, at the end of six weeks.

Potato plugs.— Potato plugs were placed in running water for three hours, and were then put into tubes containing over five cubic centimeters of distilled water (the lower end of the plugs being in the water) and sterilized for twenty-five minutes at 100° C. for three consecutive days. The streak was made with a platinum needle from a six-days-old nutrient agar culture. The tubes were kept at room temperature of about 25° C. A slightly yellow, glistening streak developed along the path of the needle in two days. It made a moderate, slightly raised, echinulate growth, covering from one-half to two-thirds of the slant in about ten days but never completely covering it. One of the strains, however, repeatedly covered the entire surface of the cylinders in from five to six days. The growth had a contoured, wet, glistening, amber yellow (2) appearance. It was slimy, was free from odor, and turned the cylinder slightly gray. Most of the strains had only a slight action on potato starch.

Bouillon.— Both meat broth and meat extract bouillon were used. The meat broth material gave an acid reaction of + 15 Fuller's scale, and the meat extract bouillon recorded + 12. Inoculations were made from a three-days-old bouillon culture. All cultures were kept at about 25° C.

The medium was clouded after from twenty-four to forty-eight hours. An amber yellow (1) rim formed in from twelve to fifteen days, followed by formation of a pellicle. On being shaken, the material settled in the form of yellow, viscid flakes. The material cleared in about six or eight weeks and became raw sienna (3) in color, with a slimy, amber yellow (2) bacterial growth in the bottom of the tube. Very little difference was observed in the growths of the organism in extract bouillon and in broth bouillon.

Milk.— The milk used was maize yellow (1) in color, due to overheating in sterilizing, and was about 1.8 per cent acid. All tubes were inoculated with a one-millimeter loop of beef broth culture three days old, and kept at 25° C. Two days after inoculation there was a slight whey ring at the surface of the milk; after four days the ring was three or four millimeters deep. Smith (1903) states that this organism slowly precipitates casein, which is then gradually redissolved.

In about ten days the whey ring was five or six millimeters deep and was amber yellow (3) in color, and the fluid curd gradually changed to yellowish tan (4) in color. The amount of clear whey gradually increased, and in about twenty-five days it became gamboge yellow (2) and the curd gradually became sienna (4) in color. The whey was viscid, while the mobile precipitate finally became a compact, slimy mass. An amber yellow pellicle was formed in from fifteen to twenty days. Filamentary threads often extended from the pellicle to the curd below. The pellicle finally settled. On shaking tubes containing a well-formed pellicle, yellow, slimy threads sometimes extended from the surface of the medium to the bottom of the tube.

An amber yellow (2) layer of the organism soon formed on the curd, and gradually lowered in the tube as the curd dissolved. About the twenty-second day many white tyrosine crystals formed in this layer.

About the forty-fifth day the pellicle in many cases had settled. The whey now constituted three-fourths of the material, was viscid, and was saffron yellow (4) in color. The precipitate was a yellow-buff (1), slimy mass dotted with white tyrosine crystals.

In four-months-old cultures the material was about one-half dried out and the whey constituted about one-half of the material. It was Indian chestnut-red (2) in color, with a thick, slimy, fawn-colored (2) coagulum dotted with white tyrosine crystals.

Litmus milk.— In the test with litmus milk, the culture material had been incubated at 37° C. for forty-eight hours just before the inoculations were made. It was violet (4) in color, and a one-millimeter loop of a three-days-old beef broth culture was used for inoculating each tube.

On the second day a bluish violet (4) ring two millimeters deep had formed on the surface of the medium, and by the fourth day the surface ring was from six to eight millimeters deep, in many cases having an amber yellow (1) rim. The material below the bluish violet (4) ring still retained its original color, but gradually became dark violet.

In about fifteen days the top ring became pansy violet (4) in color and was from eight to ten millimeters wide. The amber yellow (4) rim was more prominent and usually a pellicle was formed. In some cases the deposit below the whey had changed to madder brown (2) in color.

By the twenty-fifth day an amber yellow (1) pellicle had formed, in some cases with filamentary threads extending to the deposit below. On shaking of the tubes, the pellicle formed into thick, slimy threads sometimes extending from the surface of the medium to the bottom of the tube. In some cases the litmus was wholly reduced by the twenty-fifth day. The whey ring was about twenty-five millimeters deep, with the upper five millimeters pansy violet (3) and the lower twenty millimeters yellow lake (3) in color. The deposit in the bottom of the tube was brownish drab (2) in color, and was covered with an amber yellow (1) layer of the organism often three to four millimeters deep. Many white tyrosine crystals had formed in this layer. In about forty-five days the pellicle had settled, leaving a dull yellow-green (1) rim. The litmus had been in some cases completely, and in others only partially, reduced, but the material in most of the tubes had returned to a darker color. The whey by transmitted light was plum-violet (4) in color. There was a thick, slimy, brown-drab (2) deposit in the bottom of the tube, about 10 millimeters deep, and on the top of this an amber yellow (2) layer of the organism. Tyrosine crystals were scattered throughout the entire slimy mass.

In four-months-old cultures one-half of the material had dried out. The pellicle had settled, and a plum-violet (3) whey ring about twenty millimeters deep covered a thick, slimy, brownish drab (2) deposit six millimeters deep in the bottom of the tube. An amber yellow (2) layer of the organism on the top of the deposit was still rather prominent. Masses of white tyrosine crystals appeared throughout the entire slimy deposit.

Fermentation tubes.— A basal solution was made by adding two per cent of Witte's peptone to water. Six solutions were made from this, each containing one per cent, respectively, of one of the following carbon compounds: glycerin, saccharose, mannite, dextrose, maltose, lactose. Five fermentation tubes were filled with each of these solutions and sterilized by heating for twenty-five minutes on three successive days. Three tubes of each set were inoculated and two were left as a control. The inoculated tubes each received a one-millimeter loop of culture material from a five-days-old peach bouillon culture.

Five days after inoculation a cloudiness appeared in the open arm of each of the inoculated tubes. This turbidity increased with age, extending more than halfway into the U in the dextrose, saccharose, and maltose cultures; but at the end of twenty-five days there was no growth in the closed arm and no gas. There was a well-defined line between the growth in the bulb and the clear liquid in the closed arm, which seemed to point strongly toward the aërobic nature of the organism. A pellicle had formed on all the dextrose, maltose, and saccharose cultures, and in most cases there was a yellow, slimy growth of the organism at the bottom of the open arm. There was very little growth in the mannite tubes.

The cultures were tested for acidity after they had grown for twenty-five days. In glycerin the acidity increased from + 0.8 to + 1; in saccharose, from + 0.9 to + 1.1; in mannite, from + 0.9 to + 1; in dextrose, from + 1 to + 2.5; in maltose, from + 0.8 to + 1; in lactose, from + 0.8 to + 1.1.

Uschinsky's solution.— In the test with Uschinsky's solution, tubes were inoculated from a four-days-old broth culture. A one-millimeter loop of the material was placed in each tube.

The tubes were all clouded after three days. The solution often became viscid in about twenty-five days. A cream yellow (1) pellicle formed in from twenty to thirty days or later. The pellicle finally settled, forming a slimy, cream yellow (3) bacterial mass in the bottom of the tube.

Sodium chloride bouillon.— A one-millimeter loop of a six-days-old, + 12, peptonized beef broth culture was placed in tubes of peptonized beef broth containing, respectively, 1, 2, 3, 4, 5, 6, and 7 per cent of chemically pure sodium chloride. Three tubes were inoculated with cultures of each kind of host — apricot, nectarine, peach, and plum. At the end of seven days clouding was apparent in all tubes containing 1, 2, and 3 per cent of sodium chloride. Both the peach and the plum organisms grew in a 3.5-per-cent salt bouillon. There was no clouding in the 4-, 5-, 6-, or 7-per-cent solutions.

Growth in bouillon over chloroform.— By means of a sterile pipette, five cubic centimeters of chloroform was added to fifteen cubic centimeters of bouillon. Fifteen tubes were shaken twice a day during the period of this test. On the eighth day the tubes were divided into five lots, four of which were inoculated with six-days-old bouillon cultures of apricot, nectarine, peach, and plum organisms, respectively; the fifth lot being used as a control. These cultures were under observation for thirty-two days, but no growth was observed in any of the tubes.

Nitrogen nutrition.— In filtered river water containing one per cent of asparagin, the organism made a slow initial growth first visible in six days. When distilled water was used the first signs of growth failed to appear before the tenth day, and in some cases as late as the fifteenth day, even when the strongest, oldest cultures were used. A very slight growth was obtained in river water containing only dextrose. River water alone failed to give any growth.

Nitrates.— Twelve tubes, each containing ten cubic centimeters of peptonized beef broth to which had been added one per cent of nitrate of potash, were divided into four lots of three tubes each. The solutions in each lot were then inoculated with apricot, nectarine, peach, and plum strains, respectively.

At the end of ten days the tubes were clouded and a tube from each lot was tested for nitrates in the following manner: To each culture one cubic centimeter of boiled starch water and one cubic centimeter of potassium iodide solution (1–200) were added. A few drops of sulfuric acid were then introduced. The peach and plum cultures gave a slight blue reaction, and on the twentieth day the remaining tubes each gave a distinct reaction. However, six months later these experiments were again repeated three times, with the same strains of the four organisms, and not a single culture gave the reaction.

Indol.— A number of tubes of Uschinsky's solution with the addition of one per cent of Witte's peptone were inoculated with a five-days-old agar culture. Apricot, nectarine, peach, and plum strains were used in making these inoculations. The inoculated tubes all showed considerable growth on the fifth day, but a test for indol was not made until the tenth day. Sulfuric acid water (1–2) and dilute sodium nitrate (1–200 in water) were used. On the tenth day all cultures gave a slight rose color after being placed in boiling water for two minutes, indicating indol production. The other tubes were tested twenty days later and a well-marked indol reaction was obtained from all the cultures.

Cultures of the four strains made in Dunham's peptone solution gave positive results at the end of twenty days. There was a trace of pink before heating, and after four or five minutes in a water bath at 80° C. the reaction became distinct.

Toleration of sodium hydroxide.— Beef bouillon having a reaction of +10 Fuller's scale was used. Sufficient sodium hydroxide was added to obtain a reaction of 0, —2, —4, and so on for every two degrees of Fuller's scale to —30. The sodium hydroxide was added just before the last sterilization. Titrations made immediately following the sterilization, while the medium was still warm, showed an apparent loss of alkalinity. In many cases, although not always, the check lots of the medium titrated on the fifth day or later gave the original reaction.

Tubes inoculated and placed in an incubator at 25° C. gave a good growth in all cultures down to —10 in forty-eight hours. There was a slight growth in the —10 cultures, and at the end of two weeks a very slight growth occurred in the —13 cultures. No growth occurred in the tubes containing larger amounts of sodium. The best growth was in cultures containing from —2 to —4 sodium hydroxide.

Toleration of hydrochloric acid.— Ordinary beef bouillon having a reaction of + 12 Fuller's scale was used. Sufficient hydrochloric acid was added to 100 cubic centimeters of the bouillon to obtain a reaction of + 14, + 16, and so on for every two degrees of Fuller's scale to + 36. There was a very good growth in forty-eight hours in all the tubes up to + 22, and a slight growth in the lot having a reaction of + 24. A very slight growth was observed on the fourth day in the medium having a reaction of + 26; this growth became slightly stronger by the tenth day. No growth occurred in any medium containing larger quantities of acid. The best growth developed in lots containing from + 12 to + 20 acid.

Resistance to drying.— Small drops of four-days-old bouillon culture were placed on sterile cover glasses in a covered sterile petri dish, and left in an incubator at room temperature of from 18° to 23° C. At intervals of twenty-four hours one of the cover glasses was transferred to a tube of sterile bouillon. Three tubes were used for each test. Growth occurred up to the eighth day, after which the tubes remained sterile.

This experiment was repeated a number of times with bouillon cultures from four to ten days old. As a rule there was no growth after the eighth day; in one case, however, growth occurred on the fifteenth, and in another case on the twenty-first, day.

Effect of sunlight.— Hiss-glucose tubes inoculated from a three-days-old bouillon culture were poured into petri dishes and placed in the bright sunlight for forty-five and sixty minutes, respectively, half of each plate being covered with black paper. After six days, on each of six plates there were numerous colonies on the covered parts but none on the exposed parts. A few colonies, however, developed on the uncovered parts of all the plates exposed for fifteen, twenty, and thirty minutes.

Effect of germicides.— Poured Hiss-glucose plates were made with the plum organism after it had been exposed to various concentrations of copper sulfate, mercuric chloride, formaldehyde, lime-sulfur solution, the self-boiled lime-sulfur mixtures, and arsenate of lead.

In a copper sulfate solution (1–10,000), colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. However, there were only one-fourth as many colonies on the five-minutes exposures as on the one-half-minute exposures. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In a mercuric chloride solution (1–150,000), colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. The colonies on the plates exposed for five minutes remained small and failed to develop the yellow color. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In a formaldehyde solution (1–300), colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. However, there were three times as many colonies in the one-half-minute exposures as in the five-minutes exposures. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In a lime-sulfur solution (15–15–50 formula) in a 1–10,000 dilution, colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. However, there were only one-half as many colonies on the five-minutes exposures as on the one-half-minute exposures. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In a self-boiled lime-sulfur mixture (8–8–50 formula) in a 1–52 dilution, colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In arsenate of lead, 1–208 dilution, colonies appeared on all plates on which the organism had been exposed for one-half, five, fifteen, and thirty minutes, respectively.

Diastatic action.— The test for diastatic action was made on potato cylinders. Twenty-eight strains of the organism were grown on potato cylinders for twenty-five days. The cultures were then tested for starch reduction, and only one of the strains showed marked diastatic action. In all the other cases the only indications observed were a slow and slight tendency toward the conversion of starch into amylodextrin, shown by a gradual change in the iodine reaction from a clear blue to a purplish tint.

These tests were made according to the directions given by Harding and Morse (1909): "These potato cultures were crushed in porcelain mortars and 50 cubic centimeters of water added to separate the particles so that the effect of the iodine could be easily seen. The presence of unchanged starch was determined by the gradual addition of a weak solution of iodine in potassium iodide. After a sufficient amount has been added to satisfy some of the other compounds which are present the starch grains are turned to a blue-black. The extent of the reduction of the starch by the bacterial growth is estimated on the basis of similar tests of uninoculated potato cylinders."

The uninoculated cylinders, when tested with the iodine-potassium-iodide solution diluted with water, always yielded a bright blue reaction. The starch reaction was also strong after the organism had been grown on the cylinders for several weeks, although a purplish color indicated a slight diastatic action.

Fragments of potato scraped from under the yellow slime from a culture forty days old, mixed with about twenty times their bulk of water, gave a purple or a blue-purple reaction. When fragments of material from the back or the center of the cylinder were used and treated in the same way, they invariably gave the blue reaction. The water below the potato cylinder, when it contained more or less of the yellow slime of the organism, also invariably gave a purplish blue reaction.

Pepsin.— A peptonizing ferment must be present, since gelatin is liquefied and casein is slowly dissolved with the formation of tyrosine.

Lab ferment.— In the milk cultures the casein is thrown out of solution without marked acid production, which indicates the presence of a lab, or rennet, ferment.

Invertase.— Cultures of all four strains of the organism were grown in filtered river water containing two per cent of chemically pure saccharose and two per cent of Witte's peptone. On the twenty-fifth day all the cultures were tested for inverted sugar with Fehling's solution, and every culture gave the glucose reaction.

Thermal death point.— Preliminary tests indicated that the thermal death point must lie between 49° and 54° C. The following tests were made to determine the point more nearly: Six sets of twelve tubes each of peptonized beef broth were inoculated with the peach organism from a five-days-old-slant Hiss-glucose culture, and the tubes were placed in water at a constant temperature of 49°, 50°, 51°, 52°, 53°, and 54° C., respectively. The tubes were removed at the end of ten minutes, and were kept at 25° C. for two weeks. At the end of five days growth had taken place in all the tubes that had been exposed at 49° and 50° C. Growth appeared also in three of the twelve tubes that had been kept at 51° C. No growth appeared at the end of fourteen days in tubes that had been kept for ten minutes at 52°, 53°, and 54° C.

The experiment at 51° and 52° C. was repeated several times with different strains of the organism, and in a few instances growth occurred in cultures kept at 51° C. for ten minutes. There was never any growth in cultures kept at 52° C.

The thermal death point, therefore, is about 51° C. Smith (1903) also placed the thermal death point of this organism at about 51° C.

Optimum temperature.— The optimum temperature appears to lie between 24° and 28° C. Growth at 24° and at 28° C. on Hiss-glucose and peptonized beef bouillon was considerably better than at 32° C. Growth at 20° and at 36° C. was about the same, but was considerably less than at 32° C. and much less than at 24° or 28° C.

Maximum temperature.— Four-days-old bouillon cultures of ten strains of the organism were used to inoculate slant Hiss-glucose medium. Six tubes were used for each strain; three tubes of each lot were placed in an incubator at 37° C., and three were kept at room temperature. All cultures, both in the incubator and at room temperature, made good growth; however, those exposed to the higher temperature were of the color of the medium, while those in the lower temperature had developed the normal yellow color. On the fifteenth day the heat of the incubator was reduced to 25° C.; on the sixteenth day one of the cultures had taken on a yellow color, and by the eighteenth day all the cultures had developed the normal color. From these results it is evident that 37° C. is close to the limit of growth for the organism on Hiss-glucose.

In a series of experiments, slant Hiss-glucose and bouillon cultures were kept at 20°, 24°, 28°, 32°, 36°, and 40° C., respectively, for three weeks. Six-days-old bouillon cultures of four strains of the organism were used for making the inoculations. Two three-millimeter loops of the fluid were used for each tube. Three tubes of bouillon and two of the slant Hiss-glucose medium were used in each set. Five tubes of bouillon and three of the Hiss-glucose medium were also inoculated and were placed at room temperature (from 23° to 25° C.), to serve as checks. All strains of the organism on both kinds of media made decidedly the best growth at 24° and at 28° C. They also made better growth in both media at 20° than at 32° C., and the growth at 32° was considerably better than at 36° C. There was no growth at 40° C.

The growth on Hiss-glucose cultures kept at 20° and at 24° C. developed the normal yellow color; tubes kept at 28° C. developed a growth a shade lighter in color; those kept at 32° C. were much lighter, and had a mere trace of yellow; in those kept at 36° C. the yellow shade had disappeared entirely, and the growth was of the color of the medium.

Loss of virulence.— Park (1905) states that " bacteria differ . . . as to the ease and rapidity with which they grow in any nutritive substance and the amount of poison they produce. Both of these properties not only vary greatly in different members of the same species, but each variety of bacteria may to a large extent be increased or diminished in virulence."

In June, 1910, four cultures, one each from apricot, nectarine, peach, and plum twigs, were obtained at Mountain Grove, Missouri. All the cultures were grown continuously on a Hiss-glucose medium. It was observed that the plum organism obtained at this time was the most vigorously growing strain in the laboratory. In the inoculation work in Missouri during 1910 it was also the most aggressive on all four hosts. In the work at Cornell University during 1911 the writer was much surprised to find that not only had this strain lost its vigor on the various culture media, but the injury was also far less marked when it was inoculated on the green tissues of the different hosts. In the fall of 1911 Abundance plum twigs were inoculated with cultures of this strain, and in the summer of 1912 the organism was regained from one of the twigs. The culture was again grown continuously on a Hiss-glucose medium. The regained culture made a slightly better growth on the various media than did the culture which had been growing continuously on the Hiss-glucose medium, and was also slightly more aggressive when inoculated on the green tissues; but it was far less virulent than when first obtained. In the summer of 1912 the four old strains which had been growing continuously on the Hiss-glucose medium since 1910 were rejuvenated by growing them in bouillon for fifteen days, making transfers to a new medium every third day, and finally on the fifteenth day all were again placed on the Hiss-glucose medium. The plum organism at once became again the most vigorous grower, and when inoculated on peach leaves and twigs it was also the most aggressive. From these results it appears that vigor is the controlling factor in the virulence of the organism.

Group number.— The group number, according to the descriptive chart of the Society of American Bacteriologists, is 211.2232523.

Life history

Inoculation.— *Bacterium Pruni*, so far as is known, passes its entire life cycle in nature within the tissue of the host. The disease usually makes its appearance in the spring shortly after the leaves have unfolded, but as a rule serious outbreaks do not occur before May and often not until later. Cankers on the twigs and limbs are the principal sources of infection in the spring. Trees bearing twigs on which inoculations result in the development of well-formed cankers, are invariably the first to develop leaf spot abundantly the following year. Young trees which suffer severely from repeated outbreaks of the shot-hole condition usually show many twig cankers. Twig cankers are also constantly associated with the early spring outbreaks on older trees. The tissues of the bud may likewise become invaded, and are often responsible for the early spring infection. Eleven out of fifty cultures made from the centers of buds taken in August from peach twigs on which the foliage was badly shot-holed, yielded many colonies of the yellow organism.

The bud cut from bud wood obtained from an infected tree often conveys the organism to healthy stock. Laboratory experiments indicate that when exposed to sun and air the bacteria which lodge on twigs and buds retain their vitality for only a very short time.

If the twigs are killed after the cankers are well formed, the organism may remain alive in the dead tissue for a considerable time. Many peach and plum twigs cross-inoculated by the writer in the summer of 1910 died in the following winter, but cultures of the organism were repeatedly obtained from the dead twigs up to the middle of the next June. From that time on it was impossible to secure cultures from this source. Cultures were readily obtained, however, from the cankers on the green inoculated twigs.

Old leaves on the ground, if well protected from sun and air, may possibly harbor the organism for some time, but it is doubtful whether they ever constitute an important source of infection. It is possible that pruned twigs and limbs left on the ground may occasionally be the source of primary infection, but in the large percentage of cases the primary infection can be traced to cankers on twigs and limbs of the trees. The writer's experiments indicate that the organism soon loses its vitality in material on the ground.

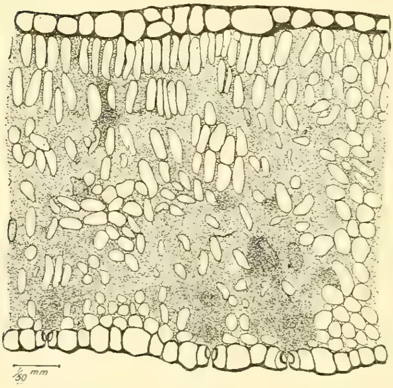

Fig. 68.—*Cross section of a peach leaf which was inoculated with a strain of Bacterium Pruni from the fruit of the plum. The material was collected eight days after the inoculation had been made. The organism entered the leaf through the stomata.* (*Drawing made with the aid of a camera lucida*)

In October, 1911, fifteen hundred peach leaves, one thousand plum leaves, and fifty peach twigs, which had been cross-inoculated with cultures of the four strains of the organisms, were put between wire screens and left over winter on the ground, where they were fully exposed to the sun. In April, 1912, all attempts to regain the organism from these twigs and leaves failed. In another experiment, however, Hiss-glucose poured-plate cultures made on June 1, 1913, from diseased leaves that had been collected on October 10, 1912, and kept in the laboratory between blotters, yielded colonies of the organism.

The organism gains entrance to the host through the stomata (Figs. 68 and 69) and the young lenticels during periods of sufficient moisture. A film of water on the surface of the leaf, the twig, or the fruit enables the bacteria to move about and gain entrance to the substomatal cavity. Inoculation ordinarily does not take place in the absence of moisture. Lenticels and stomata are fairly well distributed on the young twigs, and the stomata are more or less plentiful on the green fruit and on the lower surface of the leaves, but there are none on the upper leaf surface. Consequently inoculation may occur anywhere on the young, tender twigs and the green fruit, while on the leaves the organism can gain entrance only from the lower side.

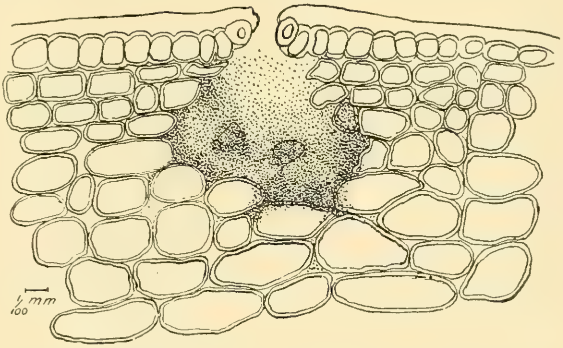

FIG. 69.—*Early stage of fruit spot on Burbank plum fruit. The fruit had been inoculated with a strain of Bacterium Pruni, isolated from a peach tree. The material was collected seven days after the inoculation was made. It was fixed in strong alcohol, infiltrated with paraffin, and cut on the microtome. The sections were stained with acid fuchsin.* (*Drawing outlined with the aid of a camera lucida*)

In 1911, at Ithaca, New York, 332 Elberta peach leaves of different ages were inoculated in the early morning. A few drops of water containing numerous bacteria in suspension were placed, by means of a pipette dropper, on the upper or the lower surface of each leaf. In all, 149 of the leaves were inoculated on the upper, and 183 on the lower, surface. In four weeks 177 of the leaves inoculated on the lower surface contained many spots; 6 were clean. All the 149 leaves inoculated on the upper surface were free from infection. In the same year 100 plum leaves were inoculated in the same way, 40 on the upper and 60 on the lower surface. At the end of five weeks, 55 of the leaves inoculated on the lower surface contained many brown spots, and 5 were free from lesions. All the leaves inoculated on the upper surface were free from spots. All the check leaves were normal.

From the results it was evident that a moist lower surface is essential for inoculation and spread of the pathogen on the leaves. Observations show that dew plays quite as important a part in the dissemination of the pathogen as does rain. Rain and dew are not only important factors for inoculation, but they also carry the bacteria to the healthy leaves, twigs, and fruit, and thus frequently serve as agents of transportation.

The bacteria are no doubt carried from tree to tree and disseminated on the young leaves, fruit, and growing twigs by insects of various kinds. Waite (1898), Stewart (1913), and others have proved that bees, wasps, flies, snails, aphids, and beetles carry the pear blight organism from oozing cankers to open blossoms and young shoots, and thus spread the blossom and twig blight of apples and pears throughout the orchard. From the large numbers of various insects often found working on the diseased foliage and fruit of trees affected with *Bacterium Pruni*, and from the nature of the bacterial ooze, there appears little doubt as to the importance of insects in the dissemination of this organism.

In spring, when the weather is cold and unfavorable for the development of the organism and the insects are sluggish, only a few primary inoculations occur and only a few scattered spots develop on the foliage. As the season advances and the weather becomes warmer, the bacteria multiply and escape from the spots of the early infections, the secondary infections gradually become abundant, and the leaves that contained only a few spots at first soon become dotted with them. Insects also become more active, and secondary spots of infection multiply rapidly.

In the inoculation experiments on plum leaves in 1913, snails and aphids were undoubtedly the chief agents in conveying the organism from the inoculated diseased leaves to the young leaves as they unfolded. The new leaves of the trees on which there were no snails nor aphids remained free from infection. Poured plate cultures were made from each of eleven snails and eighteen aphids collected from these trees. Three of the snail cultures and two of the aphid cultures yielded colonies of the organism.

The great frequency with which infection occurs on the leaves along the path taken by ants leaves little doubt that these insects also aid more or less in spreading the disease. Nevertheless, cultures from more than one hundred ants gave negative results.

As a rule, insects merely carry the virus from leaf to leaf or from tree to tree, and seldom introduce the bacteria directly into the tissue of the host.

Still the writer has frequently obtained cultures of the organism from both feeding and egg punctures of curculio on fruit, and in a number of cases it has been practically certain that the insect was the agent of inoculation.

Infected leaves are often blown about by the wind, and no doubt are sometimes responsible for the occurrence of primary infection. Yet danger from this source is comparatively slight, since the leaves soon dry out and the bacteria quickly lose their vitality. The disease usually advances in the orchard in all directions from a center of infection, often forming a more or less circular diseased area. After the organism has become established, the movement of limbs and branches caused by high wind during storms also furnishes a means of conveyance for the bacteria from diseased to healthy organs.

Old neglected trees, the plum in particular, often serve as sources of inoculum. In the past seven years two outbreaks of the disease on the Missouri State Fruit Experiment Station grounds were traced directly to infected Burbank, Abundance, and Golden plum trees.

Where the organism has become well established, diseased trees may serve as a source of inoculum and young trees planted in adjoining fields gradually become diseased. In 1906 a very striking case of this sort came under observation. A tract of one hundred and sixty acres of land on the east side of an old peach orchard at Koshkonong, Missouri, which contained many infected trees, was planted in the spring with two-years-old Elberta peach trees. In October the foliage and twigs of the trees in the ten rows next to the old trees were badly diseased. During 1907 the organism gradually advanced, and by the close of the season the trees in one hundred and ten rows were infected. The disease, however, was much worse in the first thirty rows than in the others. During 1908 the organism spread entirely across the orchard and every tree in the entire tract was injured to a greater or less extent. The trees in the first thirty rows next to the old orchard suffered most severely, and many of them were permanently injured.

Man himself is often an important agent of inoculation. In selecting material for budding, nurserymen frequently cut twigs from diseased trees, and thus, by inserting the infected bud material, inoculate the stock.

In 1907, on the Missouri State Fruit Experiment Station grounds, several thousand healthy peach seedlings were budded with buds taken from infected Elberta twigs. During 1908 the shot-hole condition developed on the foliage of nearly all these trees, and cankers were more or less plentiful on the twigs of many of them. Trees of this sort, when set out, not only grow poorly, but also serve as centers from which all neighboring trees become inoculated. In the course of the past five years several cases have come under observation in which a few infected trees from a poorly kept nursery were mixed in large shipments of clean trees. The infected trees served after planting as inoculum centers, and three years after planting every tree in these orchards was diseased.

In a careful inspection of peach and plum trees in various sections of South Carolina, not a single case of the disease was observed on isolated seedling peach and plum trees; yet the organism was repeatedly collected on the budded peach and Japanese varieties of plum in both family and commercial orchards, indicating that the nurseries are largely responsible for the spread of the pathogen.

Incubation.—The period of incubation varies more or less, depending on temperature, moisture, and method of inoculation. The period is about the same on all four kinds of trees. In some cases, however, it appears to be slightly longer on leaves and twigs of apricot and plum than on those of the nectarine and the peach.

In experiments in which the organism was suspended in water and applied to the leaves and fruit with an atomizer in warm, moist weather, the period apparently varied from seven to fifteen days, but in cold weather the period was prolonged to twenty and even twenty-five days. When water containing the organism was applied in warm weather to the lower surface of the leaf and to the fruit by means of a pipette dropper, and was spread by gently rubbing leaf or fruit between the thumb and the fingers, signs of disease appeared on the leaves in from five to seven days and on the fruit after seven or eight days; in cold weather, however, the period was considerably extended on both leaves and fruit. On the twigs, considerable difficulty having been experienced in obtaining results with the spray and rubbing method, a hypodermic syringe was used. The needle was inserted just beneath the cuticle and a small quantity of water containing the bacteria was forced into the tissue. Very uniform results were obtained in this way. The period of incubation following these inoculations varied from four to ten days, depending largely on the temperature and on the number of organisms forced into the tissue. In the twig experiments moisture did not influence the period of incubation to so marked an extent as in the experiments with leaves and fruit.

Infection.—Infection occurs soon after the bacteria pass through the stomata into the substomatal chambers, where they multiply and secrete a cytolytic enzyme which soon breaks down the cell walls of the neighboring cells, giving the organism access to an abundant food supply.

Serial sections made from infected plum fruit show that the organism at first occupies merely the substomatal chamber, but the neighboring cells are soon broken down, and as the bacteria multiply, the deeper tissues become involved. In about six or seven days a small cavity filled with bacteria is formed, and the first evidence of disease appears in the form of a water-soaked spot just beneath the stomata. A cross section through one of these spots shows the cavity filled with a compact mass of bacteria (Fig. 69, page 415). In about ten days or more, depending much on the weather conditions, the bacteria escape to the surface through the stomata or a slight central rupture. In some cases the bacteria burrow deeper and spread laterally, forming a somewhat circular, sunken, black area. The bacteria reach the surface through the various stoma and central rifts.

The tissues of the leaf are invaded in much the same way as are those of the fruit. At first the parenchyma tissues are those principally involved, but finally the vascular bundles also may be attacked (Fig. 68, page 414). The external signs of injury sometimes appear a day or two earlier on the leaves than on the fruit, and the ooze may also be evident a little earlier. The area in which the cells have been broken down assumes a more or less circular form and soon becomes separated by a line of cleavage from the healthy tissue. The injured tissue within this area soon contracts, dries out, and falls away, leaving a fairly clean-cut hole in the green tissue.

The tender tissues of the young shoots are also injured in much the same way, but the incubation period in the older tissues of the twigs is apparently longer and the development of the water-soaked spots is often slower. A cross section through one of these spots also shows a cavity filled with a compact mass of bacteria. The black-spot condition on the twig appears in from twenty to thirty-five days. Many of the phloëm cells of the sunken black area are broken down and the entire tissue is more or less disorganized (Fig. 70). Radial rifts or pockets in the young wood and cortex extend outward, often to the epidermis. As the twigs increase in size these rifts open and form an approximately longitudinal fissure. The bacteria escape through these fissures in countless numbers as bacterial ooze.

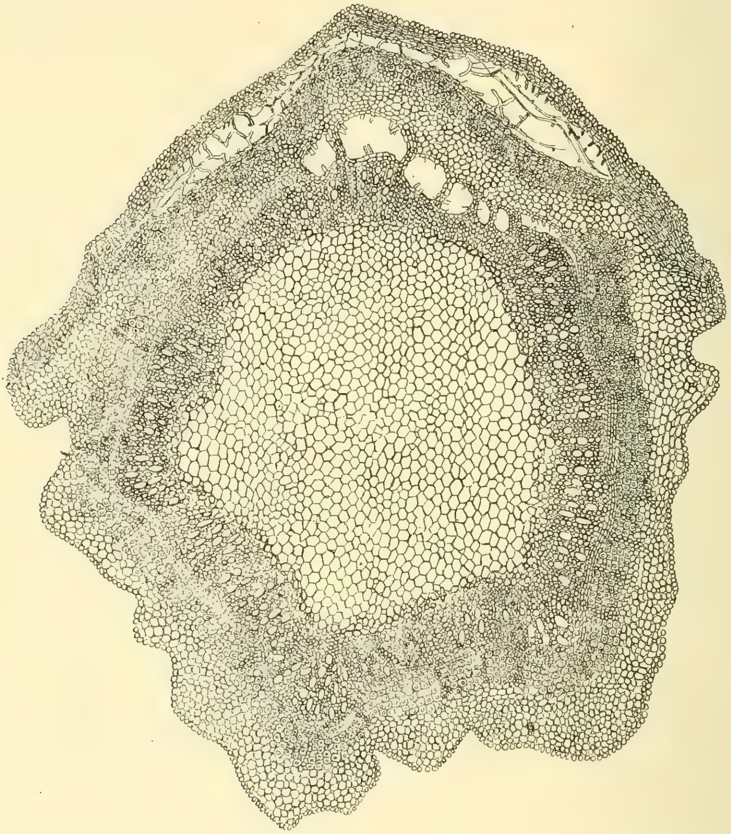

Fig. 70. — *Cross section of a young peach twig inoculated with a pure culture of Bacterium Pruni obtained from a plum twig. Bacterial pockets are most abundant in the cortical parenchyma. All cortical parenchyma cells in the infected region are distorted. The bacterial pockets in the cambium region also are to be noted. Occasionally the large ducts in the young wood are filled with the organism*

New infections occur only on the current season's growth. The organism is most aggressive in the young, tender tissues of the twigs. As the tissues harden, the influence of the organism in many cases becomes less marked and the blackened areas gradually become lighter in color; by the close of the season these areas have in many cases assumed much the same color as the old bark. During the second year a large proportion of the twig cankers thus disappear. A cross section of a twig through such cankers shows that no new bacterial pockets have appeared near the cambium zone and the old cavities have gradually been crowded into the outer bark. The organism, however, in some cases at least, is still alive and active, and no doubt often serves as a source of inoculum.

EFFECT OF ENVIRONMENTAL FACTORS

The condition of the weather is the most important environmental factor with respect to this disease. This is particularly true as regards moisture, but temperature is also of marked consequence. The shothole condition does not usually appear until about the middle of May or the first of June. In a very warm, damp spring, however, it may appear as early as the first of April, while in other years severe outbreaks may not appear until in August or even later. A temperature of from 20° to 28° C. is the best for the growth of the bacterium. Cold weather not only retards its growth, but also checks the activities of insects, which are the important agents in disseminating the organism. In the writer's spring and fall field work, it was frequently observed that when a cold period followed immediately after a series of inoculations had been made, the growth of the organism was retarded, and in some cases three, and even four, weeks passed before signs of disease appeared. The warm, slow, continued rains of the summer furnish the best conditions for the rapid spread of the disease. Heavy, driving rains of short duration followed by sunshine and winds are not favorable to its spread, since many of the bacteria are washed to the ground, and the leaves are quickly dried off and the few bacteria that may be spread will be quickly dried and killed.

The foliage of eight three-years-old peach trees, which were growing in boxes in the open, was inoculated with pure cultures of the organism. In three months the foliage on all the trees was dotted with brown spots. Four of the trees were then moved into the greenhouse and water was applied only to the roots. No new spots appeared on the old leaves after the second week, and all the new leaves that developed in the greenhouse remained free from infection, while the leaves of the four trees in the open continued to develop many new spots.

The relation of moisture to the spread of the disease is also brought out to a marked extent in the field when a favorable wet period is followed by a dry period. All the leaves formed in wet weather contain many brown spots and usually develop the shot-hole condition more or less, while those formed in a dry period remain free from spots, the contrasts in the foliage of the two periods being sometimes very striking.

Heavy dews, if the temperature does not fall too low, also furnish favorable conditions for inoculations, and observations indicate that they often play quite as important a part in the progress of the disease as does rain.

Shaded situations are favorable for the growth of the organism. Direct sunlight soon kills it. However, since the stomata of the leaves occur only on the lower surface, and the twigs and the fruit are usually fairly well shaded by the leaves, only comparatively few of the organisms ever become exposed to the direct rays of the sun.

In dry seasons injury from this organism is very slight, especially if the spring has been cold. Infections in such seasons are found only in the center of the tree, where the foliage remains moist from the dew until late in the day.

While it is true that temperature and moisture are the factors that govern the distribution of the organism, yet soil conditions, fertilizers, and cultivation have a marked influence on the extent of injury done. The soil in which the trees are growing has a considerable influence on the development of the disease. When a general outbreak occurs in an orchard, the trees on the higher, thin ridges invariably suffer more than those on the lower, more fertile land. Trees that have borne the heaviest crops in previous years invariably suffer more than do those that have yielded little or no fruit in previous seasons. Young trees planted in new land properly broken up before planting are more nearly free from the disease than trees planted on land poorly prepared. Trees properly pruned and well cultivated show less disease than do those that are not pruned nor cultivated.

VARIETAL SUSCEPTIBILITY

Observations show that the different varieties of the various hosts exhibit considerable variation in their susceptibility to the disease. The plums show the greatest variation. The majority of the American varieties suffer little, if at all, while nearly all the Japanese varieties, especially Abundance and Burbank, are severely injured, in some cases the disease resulting in the formation of deep limb and trunk cankers and in eventual death of the tree. Similar wounds are occasionally formed also on some varieties of apricot, nectarine, and peach, but they are far more abundant on Japanese varieties of plum.

The fruits of different varieties of the four kinds of hosts also show considerable variation in susceptibility. The nectarines suffer the greatest injury, the fruits of all the varieties of this host in Missouri being so badly cracked that they are usually destroyed by the various rot-producing organisms while still green. The effects of the disease on the fruits of Japanese plums, especially Abundance and Burbank, are nearly as severe as on the nectarines. The greater proportion of the fruits are badly distorted and cracked and the small number that are not rotted before they ripen are so badly injured that they are worthless for market. As a rule, apricot fruits are injured to a less extent than those of the nectarine or plum, but the variety Royal is severely affected. Though the fruits of some varieties of peaches, particularly Elberta, are severely injured, they are as a rule less badly disfigured than are the fruits of the nectarine or the Japanese varieties of plum (Fig. 63c).

In addition to this variation in susceptibility of the different varieties, there is also considerable variation in the amount of injury to the fruits of a given variety during different seasons. The real significance of these latter variations is not always easy to explain, but in most cases the age of the fruit and the weather conditions determine largely the percentage of infection.

In table 1 is given a list of the apricot, nectarine, peach, and plum trees in the variety orchard of the Missouri State Fruit Experiment Station, showing the nature and degree of susceptibility of the different varieties. These figures represent the average record for the year 1909. An estimate of the percentage of infection on leaves, twigs, and fruit was made by passing through the orchard, estimating and recording the injury. After the estimates had been made several trees in different parts of the orchard were carefully examined and the actual percentage of infection was obtained by counting the diseased leaves, twigs, and fruits. The difference found between the actual and the estimated percentages of infection formed the basis for correction of all the varietal estimates. Three estimates were made in the course of the summer, the first in the middle of July, the second August 5 to 7, and the third September 26 to 29. The average result of the three records is given here as the record for the year.

TABLE 1. Relative Susceptibility of Different Varieties of Apricot, Nectarine, Peach, and Plum to Bacterium Pruni*

Apricot

Bread, 40-30-30-f
Moorpark, 40–20–0–f
Peach, 75–30–0–a
Russian, 30–10–?–a
Superb, 20–15–0–p
Sweet Russian, 40–20–30–p

Nectarine

Coosa, 45–15–100–a
Munson's Cling, 50–8–85–a
New White, 58–8–100–p
Stanwick, 65–15–100–a
Victoria, 60–10–100–a

Peach

Alexander, 25–15–5–p
Alice Hampton, 20–15–35–p
Alton, 40–20–80–a
Amsden June, 8–5–25–f
Arkansas Beauty, 35–15–50–a
Arkansas Mammoth, 50–10–50–f
Arkansas Traveler, 40–10–0–a
Arnett, 55–10–?–p
Arnett No. 6, 65–5–?–a
Athens, 40–3–30–a
Banner, 15–8–35–n
Barber, 8–5–0–f
Barnard Early, 35–10–60–a
Barnes, 5–3–0–f
Beauty, 30–10–50–a
Belle (Belle of Georgia), 15–3–25–a
Bell's October, 20–10–25–n
Bequette Cling, 23–3–45–p
Bequette Free, 40–10–70–a
Bernice, 35–10–25–f
Bilyeu, 60–10–100–a
Bishop, 18–18–0–f
Blanchard, 55–10–45–a
Blood Cling, 45–10–0–p
Blood Free, 50–10–30–p
Blood Leaf, 55–5–45–a
Bokhara, 0–10–40–n
Bonanza, 45–15–60–p
Brandywine, 6–13–40–f
Bray's Rareripe, 25–15–30–f
Brett (Mrs. Brett), 20–30–30–a
Brigdon, 40–10–20–a
Briggs (Briggs' May), 18–18–40–f
Bronson, 30–15–25–a
Burke, 20–10–35–p
Carman, 60–0–30–p
Carpenter (Carpenter Cling), 35–5–50–a
Chairs (Chairs' Choice), 30–20–35–a
Champion, 60–0–30–p
Champion Yellow, 15–5–30–f
Charlotte (Early Charlotte), 35–10–?–p
Cherokee, 25–20–45–a
Chili (Hills' Chili), 20–10–30–p
Chilow, 25–40–25–p
Chinese Cling, 45–15–30–a
Chinese Free, 45–5–55–a
Christiana, 30–5–25–a
Cloves, 43–3–50–p
Cobler, 25–15–20–p
Coleman, 20–10–30–p
Collridge Favorite, 30–10–30–a
Columbia, 35–15–40–a

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*The first number following the name of the variety indicates the percentage of infected leaves; the second, the percentage of defoliation; the third, the percentage of infected fruit (" ? " in the third place indicates that the tree bore no fruit). The letter in the fourth place indicates the number of twig cankers observed: a, abundant; p, plentiful; f, few; n, none. The letter in the fifth place (in the plum varieties) indicates the amount of injury due to trunk and limb cankers: e, extreme injury causing the death of the trees; m, marked injury; s, slight injury; n, no injury.

Peach (*continued*)

Conkling, 13–8–0–f
Connecticut, 43–3–60–p
Connet (Connet's Early), 15–10–60–f
Cora, 80–8–10–a
Crosby, 13–3–0–f
Crothers, 60–0–100–a
Davenshire, 40–20–0–a
Davidson, 35–20–50–p
Dawson's Early, 25–2–0–f
Delaware, 45–5–60–f
Deming (Deming's September), 60–10–40–a
Denton, 55–15–45–a
Dewey (Admiral Dewey), 20–10–0–p
Diamond, 50–20–30–a
Dover, 45–5–70–a
Early Barnard, 10–20–0–n
Early Crawford, 10–5–25–f
Early Heath, 35–5–25–p
Early Michigan, 30–5–80–a
Early Rivers, 30–15–40–f
Early York, 40–10–50–a
Ede (Captain Ede), 15–10–25–p
Edgemont Beauty, 11–5–25–p
Elberta, 80–30–45–a
Elberta Cling, 60–10–53–a
Eldred, 65–15–35–a
Emma, 50–15–50–a
Engle (Engle's Mammoth), 15–10–0–f
Etna, 20–10–25–p
Eureka, 45–10–55–a
Family Favorite, 80–10–70–p
Fischer, 65–5–100–p
Fitzgerald, 35–10–35–a
Flatters St. John, 65–5–80–a
Flemer's Cling, 65–5–98–a
Flewellen, 35–5–33–a
Floyd, 50–10–40–a
Ford's No. 1, 50–0–70–p
Ford's White, 40–10–25–f
Foster, 30–20–30–p
Fox (Fox Seedling), 18–8–35–a
Frances, 30–10–50–a
Froth's Early, 65–15–55–a
Future Great, 20–10–25–p
Garfield, 13–8–35–p
Geary's Hold-on, 28–8–25–p
General Taylor, 5–5–95–p
Gilman's Favorite, 50–10–45–a
Globe, 15–10–50–p
Gold Drop, 21–10–60–p
Gold Dust, 30–75–30–p
Golden Gate, 50–20–?–a
Gold Medal, 40–10–30–a
Gold Mine, 30–10–35–p
Gordon, 20–15–30–f
Grand Reporter, 55–15–50–a
Hale Early, 38–8–40–p
Heand's Beauty, 60–10–45–a
Heath (Heath Cling), 60–20–60–a
Heath Free, 60–20–25–a
Henrietta, 8–5–40–f
Hiley, 26–1–40–p
Hill's Madeira, 25–5–40–p
Hobb's Late, 15–10–22–f
Hobson, 30–25–?–a
Holderbaum, 20–5–25–f
Honi Chirf, 35–10–30–p
Hoover's Heath, 20–10–10–n
Horton's River, 45–15–70–p
Hughes I. X. L., 30–10–0–p
Hyde's Yellow, 20–5–20–f
Hynes (Hynes' Surprise), 20–10–30–f
Indian Blood Cling, 10–10–0–n
Indian Blood Free, 50–10–40–a
Ingold (Lady Ingold), 30–20–90–a
Japan Blood, 55–5–0–a
Jaques (Jacques' Rareripe), 15–15–25–f
Jennie Worthern, 35–10–30–a
Juno, 45–5–30–a
Kalamazoo, 10–8–0–f
Kerr (Jessie Kerr), 25–10–0–f
Krummel's October, 20–10–45–p
Late Crawford, 40–10–40–a
Late Elberta, 60–15–35–a
Laure Cling, 35–5–30–a
Lemon Cling, 12–10–0–f
Lewis, 0–10–50–n
Lodge, 40–10–60–a
Lola (Miss Lola), 25–10–40–f
Lorentz, 33–8–0–p
Louise (Early Louise), 35–15–0–a
Lovell No. 3, 50–0–?–a
McCallister, 20–15–?–f
McIntosh, 13–8–0–p
Madison County Mammoth, 40–10–70–a
Magnum Bonum, 35–30–25–a
Mamie Ross, 20–10–40–a
Mammoth Cling, 75–15–50–a
Marshall, 30–10–30–p
Mathews (Mathews' Beauty), 50–20–50–a
Mena, 40–10–?–a
Michigan, 10–5–0–n
Mid September, 23–8–25–p
Mint's Free, 30–20–80–p
Mixon Late, 75–5–30–a
Moore's Favorite, 20–15–50–a
Morris Late White, 50–10–30–p
Mountain Rose, 25–15–70–p

Peach (*concluded*)

Muir, 25–15–30–a
Munson's Cling, 65–15–100–a
Munson's Free, 60–10–70–a
Nanticoke, 23–8–30–f
Nelson's No. 5, 40–10–45–a
New Prolific, 10–15–25–f
Niagara, 45–5–40–p
Nicholson's Smock, 40–10–40–p
October Free, 50–10–33–a
Olden No. 1, 40–10–25–p
Olden No. 3, 50–10–100–a
Oldmixon Cling, 55–15–30–a
Oldmixon Free, 50–20–35–a
Oldmixon Improved, 30–15–45–p
Orange Cling, 40–20–35–p
Oriole, 30–3–55–p
Oritz, 28–18–40–a
Oscar, 63–8–70–a
Ozark Queen, 20–20–40–p
Pallas, 43–3–70–a
Pearce's Yellow, 30–10–0–p
Peento, 5–5–?–n
Pendleton, 40–10–25–a
Perry's Red Cling, 35–15–?–p
Plaut, 50–10–35–a
Pond's Seedling, 70–10–40–a
Pool's Favorite, 8–3–0–f
Queen, 40–20–50–p
Rasin Cling, 35–25–60–p
Ray, 20–5–30–p
Red Heath, 35–5–25–a
Red River, 60–15–30–a
Reeves Yellow, 20–15–0–f
Reynolds, 18–8–25–f
Ringgold, 40–10–40–p
Rivers, 58–8–25–a
Robert, 50–20–30–a
Rodgers, 20–5–45–p
Rose of Baltimore, 48–8–30–a
Rush's Favorite, 30–10–65–a
Russell, 23–20–20–n
St. John, 50–20–80–a
Salway, 15–15–35–f
Schofield, 60–0–40–a
Scott's Nonpareil, 65–15–40–a
Sea Eagle, 30–10–30–f
Sea Eagle Improved, 25–10–30–f
Shipler, 42–15–60–a
Shoemaker, 20–2–0–n
Smock (Smock's Free), 50–20–50–a
Sneed, 70–25–40–a
Snow, 35–10–25–a
Snow Free, 30–5–45–p
Snow's Orange, 23–10–0–n
Springfield, 50–10–40–p
Steadley, 30–10–35–p
Stevens, 60–10–35–a
Stevens Late, 20–20–40–f
Stinson, 65–15–65–a
Strout's Early, 15–10–?–f
Stump, 15–10–40–p
Success, 30–10–20–p
Summer Snow Cling, 40–10–55–a
Superb, 40–20–40–p
Sussex's Late, 35–5–45–p
Sylphide, 33–13–23–p
Texas (Texas King), 10–5–65–f
Thurber, 15–3–30–f
Tillotson (Early Tillotson), 10–12–0–n
Toledo (Early Toledo), 35–5–0–p
Topaz, 15–15–50–a
Tornado, 30–10–60–p
Trebout, 50–20–70–a
Van Buren, 30–3–?–p
Waddell, 60–10–45–a
Wager, 30–15–25–n
Walker (Walker's Variegated Free), 20–15–30–p
Ward's Late, 65–15–40–a
Washington Cling, 60–10–50–a
Waterloo, 20–0–40–p
Webb's Golden, 50–5–70–f
Wheatland, 40–10–?–a
Wheeler's Late, 75–5–25–a
Wilkins' Cling, 38–8–45–a
Willett, 50–15–30–a
Williams' Favorite, 40–10–23–p
Wilson's No. 14, 45–5–?–p
Wonderful, 35–15–0–n
Worth, 55–15–40–a
Wright, 5–3–30–n
Yellow Rareripe, 25–15–0–f

Plum

Abundance, 100–60–100–a–e
America, 40–20–20–p–s
American Eagle, 55–15–10–a–e
Apple, 90–40–?–a–m
Arkansas (Arkansas Lombard), 0–0–0–n–n
Assiniboia, 55–20–?–p–n
Best of All, 45–15–0–a–n
Broadshow, 0–0–?–n–n
Burbank, 100–60–100–a–e
Case Gold Drop, 0–0–?–n–n

Plum (*concluded*)

Chabot, 85–15–100–a–m
Clifford, 0–0–10–n–n
Cumberland, 20–0–0–a–m
Downing (Charles Downing), 55–20–0–n–m
Diamond, 0–0–?–n–n
Douglass, 80–20–0–a–m
Damson (Blue Damson), 0–0–?–n–n
Elapa, 55–20–0–n–n
Funk, 0–0–?–n–m
Gonzales, 95–30–?–a–m
Grand Duke, 0–0–?–n–n
Guthrie Topaz (Topaz), 63–25–?–n–n
Hale, 90–35–?–a–m
Hattankio (Hytankio), 50–15–10–a–m
Hawkeye, 50–15–0–p–n
Hungarian Prune, 0–0–?–p–n
Italian Prune, 0–0–0–n–n
Jefferson, 0–0–?–n–n
Jones Special, 75–25–30–n–n
Kerr, 60–25–?–a–m
Krap, 55–33–0–p–n
Kroh (Poole's Pride), 40–20–10–f–m
Lacy, 100–45–?–n–n
Lombard, 0–0–?–n–n
Maryland, 0–0–0–n–n
Milton, 0–0–0–n–n
Monarch, 50–20–?–p–n
Newman, 40–20–10–f–n
October (October Purple), 85–40–?–a–m
Ogon, 95–30–30–a–e
Orleans (Smith's Orleans), 0–0–?–n–n
Owanka, 55–15–?–n–n
Peter's Gage, 0–0–?–n–n
Pond (Hungarian Prune), 0–0–?–p–n
Pride (Shipper's Pride), 30–12–?–f–n
Purple Leaf, 90–35–?–a–m
Quaker, 45–15–50–p–m
Reagan, 55–25–0–n–n
Red Goose, 40–20–?–n–m
Red June, 95–25–100–a–e
Red Nagate, 100–50–?–a–m
Red October, 55–10–0–a–m
Reine Claude, 40–8–?–p–n
Robinson, 40–15–100–n–m
St. Martin (Coe's Late Red), 0–0–?–n–n
Shropshire (Shropshire Damson), 0–0–?–n–n
Sophie, 40–10–10–f–n
Stella, 95–55–?–p–n
Tokeya, 40–10–?–n–n
Wakapa, 55–15–?–n–n
Ward October, 40–10–20–p–m
Ward October Red, 45–15–0–a–m
Wastesa, 75–35–?–p–n
Whitaker, 0–0–0–n–m
Whiting, 0–0–?–n–m
Wildgoose, 40–10–0–n–m
Willard, 100–40–?–p–e
Winnipeg, 55–25–?–f–s
Wohanka, 50–25–?–n–n
Yellow Egg, 0–0–?–n–n
Yosemite, 90–30–10–a–e
Zekanta, 48–15–?–n–n

CONTROL

Observations and experiments in control were made principally with peaches and plums, as only a few varieties of apricots and nectarines were available for experimental work. This phase of the subject may be discussed under four heads: exclusion, eradication, protection, and immunization.

EXCLUSION

In budding, the greatest care should be exercised in selecting material only from trees known to be free from the organism. Even though the twigs are free from cankers, the organism may be transferred to the healthy stock through infected buds. Laboratory and field experiments made at the Missouri State Fruit Experiment Station show that it is impossible to determine from the external appearance of the bud whether it is infected.

In preparing to set out an orchard, great care should be used in ordering the trees. Purchases should be made only from reliable nurserymen, and, if possible, the nursery should be visited and the trees inspected in the fall, while they are still in full leaf, in order to make sure that the foliage is free from shot-hole condition and the twigs are free from cankers. If this is impossible, the trees should be carefully examined for black cankers in the spring, before they are set out. The disease is usually more easily detected in fall than in spring. All infected trees should be rejected.

Since the organism is readily carried from one tree to another, the location of a young orchard or nursery in relation to old, diseased trees or orchards is of considerable importance. The writer has frequently observed old orchards which served as centers of inoculum for young trees.

In 1906 a tract of one hundred and sixty acres of new land near Koshkonong, Missouri, which bordered an old peach orchard on the south, was set with two-years-old Elberta peach trees. During the first year the foliage on the trees in the first fifteen rows became more or less diseased, but during the second year the disease advanced more rapidly and the foliage on trees in the two hundred and fifty rows next to the old orchard became involved. The trees beyond this, however, were normal and were making satisfactory growth. At the end of the third year nearly every tree in the entire orchard was diseased. Those in the first fifty rows bordering the old orchard contained more twig cankers than did those in any other part.

ERADICATION

After the disease has once become established, the chief point of attack is, of course, the cankers on twigs, limbs, and trunks of the trees, since these wounds not only serve to carry the organism through the winter but also in many cases tide it over unfavorable periods in summer. Careful pruning not only removes many of the twig cankers — and very often the cankers near the tips of the twigs contain the greatest numbers of bacteria — but also serves to stimulate new growth which often shows considerable resistance to the attacks of the organism. It is of course impossible to remove all the twig cankers in a crop season. But in an off year, when the fruit buds have all been winterkilled, the twig cankers on the old trees can be removed by cutting back the limbs to one-fourth of their entire length and carefully trimming out all the one- and two-years-old twigs from the center of the trees. Lesions on the limbs and trunks soon form rough, open cankers and can easily be found. Such wounds must also be cut out, or, if this is impossible, the diseased tissue must be completely removed by cutting around the canker well into the healthy part of the limb or the trunk. In every case the wound should be thoroughly disinfected with corrosive sublimate solution, one part to one thousand parts of water. The organism can usually be eliminated in this way on the apricot, the nectarine, and the peach. In the case of the plum, however, especially on the Japanese varieties where the organism has invaded the large limbs and the trunk, it is practically impossible to save the tree.

In 1907 the peach trees in the Missouri State Fruit Experiment Station variety orchard were heavily pruned. All the limbs were cut back one-fourth of their length. One hundred and thirty Elberta trees of the same age were not severely pruned but were treated in every other way as the other trees. During the fall the foliage on the pruned trees was of a better color and more nearly free from disease than that on the unpruned trees. In 1908 little or no injury was observed on the pruned trees, while foliage on all unpruned Elberta trees was more or less diseased. Twenty per cent of the leaves finally fell, and twig cankers also were plentiful. Chandler,[4] of the University of Missouri, has also observed in his work that severe pruning tends to make the trees more nearly free from the shot-hole disease.

PROTECTION

Spraying

Since bordeaux mixture has been used successfully in controlling various plant diseases, and lime-sulfur has been used also with more or less success, a number of experiments were undertaken in order to determine the effectiveness of these mixtures in controlling this organism.

In 1908, thirty-two eight-years-old Elberta peach trees were sprayed seven times with bordeaux mixture, and twelve were used as checks. The first application was made with the 6-4-50 formula on March 12, just before the fruit buds opened. The other six applications were made with the 2-9-50 formula after the leaves had unfolded; the first of these was made on April 21, and the others followed at intervals of from twelve to fifteen days. On August 12, the leaves, the twigs, and the fruit on the sprayed trees were free from disease. The fruit was of excellent color and quality, but there was sixty-one per cent less fruit on these trees than on the checks. The leaves on the checks were badly shot-holed and about ten per cent of them had fallen. The twigs also contained many blackened cankers. Sixty-three per cent of the fruit was infected.

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[4] Chandler, W. H., in a letter dated September 26, 1912.

In the same year, twenty-nine eight-years-old Elberta peach trees were sprayed seven times with lime-sulfur. Eleven trees were used as checks. The first application was made with the standard 15-15-50 formula on March 12, just before the buds opened. The other six applications were made with self-boiled lime-sulfur, 12-8-50 formula, after the leaves had opened; the first of these was made on April 21, and the others followed at intervals of from twelve to fifteen days. On August 12 the foliage on the sprayed trees was more or less dotted with diseased spots. The twigs also showed a few black cankers. Fifty-two per cent of the fruit contained many small bacterial wounds, but otherwise it was in good condition (Fig. 64). The yield was reduced twenty per cent. On the check trees the foliage was badly injured and ten per cent of the leaves had fallen. The twigs also showed many cankers. Sixty-three per cent of the fruit was diseased and cracked, its market value being considerably reduced.

In 1909, twenty-two nine-years-old Elberta peach trees were sprayed four times with peach bordeaux mixture, 2-9-50 formula. Twelve trees were used as checks. In 1908 curculio punctures made it difficult to determine the exact extent of injury due to the attack of this organism, and therefore in all the spraying experiments in 1909 arsenate of lead was added to the mixture used in the first two applications. The poison was added at the rate of two pounds to fifty gallons of the spray mixture. The first application was made about seven days after the petals had fallen, and the three others followed at intervals of four weeks. On August 8, as far as could be observed, the leaves, the twigs, and the fruit were free from disease. The foliage on the check trees was badly injured, and fifteen per cent of the leaves had fallen. The twigs contained many black cankers. Sixty-five per cent of the fruit was injured, and most of this was worthless.

In 1909, twenty-four nine-years-old Elberta peach trees were sprayed four times with self-boiled lime-sulfur, 12–8–50 formula. Arsenate of lead was added to the first two applications at the rate of two pounds of the poison to fifty gallons of the mixture. The first application was made about seven days after the petals had fallen, and the three others followed at intervals of four weeks. On August 8 only a few spots were observed on the leaves, and the twigs were apparently free from cankers, but fifteen per cent of the fruit was infected. The foliage on the check trees was badly injured, and fifteen per cent of the leaves had fallen. Many of the twigs were badly cankered. Sixty-three per cent of the fruit was infected, and most of this was worthless.

Apparently the arsenate of lead improved the effectiveness of the self-boiled lime-sulfur solution. It is quite probable, however, that the beneficial results obtained from the addition of arsenate of lead are due rather to the control of insects which serve as agents of dissemination than to the improvement of the mixture as a fungicide. Laboratory tests show that arsenate of lead used at the rate of two and one-half pounds to fifty gallons of water has very little influence on the growth of this organism.

From these and other experiments, it is evident that bordeaux mixture will control the disease. But the foliage of the trees, especially in the case of peach and nectarine, is extremely sensitive to the action of copper, and in some cases the trees are defoliated even when the copper is used at the rate of one-half pound of copper sulfate to nine pounds of lime and fifty gallons of water. Self-boiled lime-sulfur alone is much less effective, and in all the writer's experiments it failed to control the disease; but when two pounds of arsenate of lead were added to fifty gallons of the mixture it became much more effective.

Cultivation

A tract of about two hundred acres of new land at Koshkonong, Missouri, was set in 1906 with two-years-old Elberta peach trees which were more or less infected with the organism. Strips about four feet wide were plowed across the field and trees were set on the plowed land. The orchard was fairly well cultivated during the first three years. The organism gradually spread from the diseased to the healthy trees, and at the end of the third year every tree in the entire tract was more or less infected. Many of the trees were stunted and some of them finally died. Their death, however, could not be attributed directly to the work of this organism, but was apparently due to a combination of winter injury of the roots, premature defoliation due to the work of the bacteria, and, finally, the invasion of the roots by one of the root rot organisms. The trees of this orchard were uneven and produced very little fruit.

In the same year another tract of about one hundred acres of new land was planted with two-years-old Elberta trees brought from the same nursery. In this case the ground had been thoroughly prepared and the orchard was carefully cultivated during the following three years. A number of infected trees were observed in different parts of this orchard in the first year, but the disease spread very little and by the end of the third year all traces of disease had disappeared. The trees developed uniformly and produced large crops of excellent, clean fruit.

Diseased trees that have been properly cultivated invariably hold their leaves longer and resist the influence of the organism much better than do poorly cultivated trees. The general vigor of the host plays an important part in controlling the disease. Trees standing in thin, poor soil invariably suffer more severely than do those standing in more fertile places. Trees that yield abundantly are less resistant than those that produce little or no fruit. The foliage of infected trees that yield full crops in two consecutive years usually falls prematurely if the trees are not liberally fertilized and well cultivated.

In the spring of 1907 the peach trees in the Missouri State Fruit Experiment Station variety orchard were cut back severely, and about the middle of May complete fertilizer at the rate of 800 pounds to the acre was applied to the soil on one-half of the orchard. The same amount was applied also to an acre of Elberta peach trees of the same age which had not been cut back. Both the variety orchard and the Elberta orchard were finally planted in cowpeas. On September 1 the foliage on the trees that had been cut back and fertilized was in good condition and no premature defoliation was observed. The foliage on the Elberta trees, which had been fertilized and not cut back, was in fair condition, but from two to five per cent of the leaves had fallen. The foliage of the trees that had been cut back and not fertilized was more or less injured and from ten to twenty per cent of the leaves had fallen. The most striking results, however, appeared in September, 1908. The trees in the fertilized and cut-back block were practically free from disease. The foliage on the Elberta trees, which had been fertilized and not cut back, was more or less diseased, twenty per cent of the leaves had fallen, and many of the twigs contained black cankers. The foliage on the block of trees which had been cut back and not fertilized was much improved, not over two or three per cent of the leaves had fallen, and the twigs on very few of the trees contained cankers.

Chandler,[5] in a series of commercial fertilizer experiments conducted at Brandsville, Missouri, from 1910 to 1912, also has shown that the organism can be controlled to a large extent by the use of fertilizer. He obtained excellent results in using from 700 to 800 pounds of complete fertilizer per acre on mature trees and from 300 to 400 pounds on trees from two to three years old. Applications were made once a year in early spring or in June. Those made in June apparently gave better results than those made in spring. Chandler's results show that nitrate of soda is the essential element for controlling the disease. All plots that received nitrate of soda, whether alone or in combination with either potassium chloride or acid phosphate or both, showed very great resistance to the disease, but the plots receiving only potassium or phosphorus or both did not show any increased resistance. In the plots treated with nitrate of soda a few leaves were affected, yet very few fell as the result of the disease and the foliage had a healthy color. In some cases the trees of the check plot and those treated with potassium chloride and acid phosphate lost as high as fifty per cent of their leaves.

An application of manure well worked into the soil gives excellent results and in some cases the improved condition of the trees is more permanent than when commercial fertilizer alone is used. Good results have been obtained by seeding down the orchard permanently to red clover after a liberal application of manure. The clover is cut from time to time and allowed to rot under the trees. This practice, however, has disadvantages and cannot be recommended for general use. Very good results have been obtained also by following an application of manure with cowpeas. The tops are not cut but are allowed to stand until killed by frost, and the following spring the dead material is carefully worked into the soil. After the first year commercial fertilizers and cowpeas are used, but if the trees show marked improvement nitrate of soda is omitted from the fertilizer, since the cowpeas as a rule furnish sufficient nitrogen. This method gives good results. The peas, however, furnish an abundant food supply for mice, which are likely to injure the trees during the winter.

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[5] Chandler, W. H., in a letter to the writer dated September 26, 1912, containing a summary of some of his commercial fertilizer experiments with peach trees. A detailed account of the experiments will finally be published by the Missouri Experiment Station, Columbia, Missouri.

IMMUNIZATION

No doubt much can be accomplished in the way of resistance by crossing the less resistant with the more resistant varieties. This is especially true in the case of plums.

RECOMMENDATIONS FOR CONTROL

Since the organism is spread largely in the nursery, great care should be exercised in selecting trees for planting. Stock should be bought only from reliable nurserymen, who should be required to guarantee the trees to be free from the disease. A single diseased tree may cause serious loss after the trees are set out.

Old, neglected, budded apricot, nectarine, peach, and plum trees, especially plum, usually harbor the organism and become a menace to young orchard trees. All such trees should be cut down before the young trees are set out.

The ground must be thoroughly plowed before the young trees are set, and about one pound of a complete fertilizer should be worked into the soil about the tree while it is being set. Too much care cannot be given to the cultivation of an orchard, especially during the first two or three years.

Where the organism has become thoroughly established, systematic pruning, cutting out, and careful removing of all the diseased tissue from the cankers on the limbs and the trunks of the trees will eliminate the source of infection to a large extent. Complete eradication on old trees (seven or eight years old), however, can be accomplished only by pruning the trees. Pruning not only tends to eliminate the source of infection, but also serves to stimulate new growth which is more resistant to the disease.

Where the trees have been heavily pruned, a thorough application of bordeaux mixture (4 pounds of copper sulfate, 4 pounds of lump lime, and 50 gallons of water) just before the buds open will be of considerable assistance in controlling the disease on the fruit, especially if this application is followed by two or three applications of arsenate of lead (2½ pounds of arsenate of lead, 4 pounds of lump lime, and 50 gallons of water) to control the curculio and other insects, which aid materially in disseminating the organism. The first application of arsenate of lead should be made just as the shucks slip; the second, two weeks later; and the third, about two weeks after the second.

The older trees that are in good condition are as a rule not seriously injured by the disease. Thorough cultivation of an orchard and the use of fertilizers are absolutely essential. Complete fertilizer, at the rate of about 600 pounds to the acre in early spring for mature trees and from 150 to 300 pounds for young trees, gives very good results. In crop seasons a second application of about 250 to 300 pounds of the fertilizer should be made in June; the amount of nitrate of soda in this application should be reduced by about one-half. A liberal application of stable manure in the spring, well worked into the soil, will also give excellent results. Close attention to pruning and to the chemical and physical conditions of the soil will practically control this disease.

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LEWIS KNUDSON

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INFLUENCE OF CERTAIN CARBOHYDRATES ON GREEN PLANTS

INFLUENCE OF CERTAIN CARBOHYDRATES ON GREEN PLANTS[1]

Lewis Knudson

The classical investigations of Priestley, Ingenhouz, de Saussure, and others, near the beginning of the nineteenth century, established the fundamental facts relative to the utilization of atmospheric carbon dioxide by green plants. Yet, despite the discoveries made by these men and by others who followed, the belief prevailed in the botanical, chemical, and agronomic sciences that the source of the plant's carbon was the brown or blackish organic material of the soil, which was designated humus. Liebig (1847),[2] by his arguments based on theoretical rather than on experimental grounds, contributed largely to the relegation of this view. He asserted that the plant derived its carbon entirely from the carbon dioxide of the air. Boussingault (1860) demonstrated by experimental methods the ability of the plant to develop when organic matter was entirely wanting in the substratum. His experiments demonstrated not only the ability of the plant to procure its carbon from the air, but also the fact that humus is not necessarily a source of nitrogen, the contrary view having been formerly very generally believed. The work of Sachs, Knop, and many others subsequently, with water cultures, proved conclusively the ability of the plant to develop independently of the presence of humus.

Nevertheless the humus theory was revived from time to time, notably by Bréal (1894). An explanation was sought for the generally recognized superiority of stable manure as a fertilizer, but no adequate experimental evidence was produced in favor of the humus theory. The interest now, however, is not confined merely to the narrower aspect of the plant's relation to humus, a substance of indefinite character, but is concerned rather with the relation of the plant to dissolved organic substances that may occur in soils. One of the phases of this subject — namely, the injurious effect of soil organic substances — has received and is still receiving much attention from the United States Bureau of Soils. Practically no consideration, however, has been given in this country to the possibility of a favorable influence of dissolved organic matter, although Schreiner (1913) does suggest the direct utilization of organic nitrogenous substances. The relatively recent investigations have proved the ability of the higher plants to directly absorb, by means of their roots, a considerable number of different organic substances present in the nutrient media. This has created a new interest in the rôle of the organic material of the soil in the nutrition of the higher plants.

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[1] Contribution from the Laboratory of Plant Physiology, Cornell University.

[2] Dates in parenthesis refer to bibliography, pages 71 to 75.

It is of course recognized that the plants may grow and mature in the absence of organic material from the substratum, and it is furthermore recognized that in many soils there is such a relatively small amount of organic matter that its significance as a source of carbon would be very slight and perhaps of no direct value. However, the fact that in many soils a rich microbial flora exists, postulates the presence of directly available organic substances or of substances made available by extracellular digestion. Since there is no reason to assume that permeability or metabolism in fungi and bacteria differs fundamentally from that in higher plants, it is logical to conclude that in general what is available for the fungus is likewise available for the higher plant. In this connection the fact is significant that there exist among the phanerogams plants devoid of chlorophyll, which necessarily derive all their organic material from the soil.

Theoretically there is no reason why higher plants should not absorb dissolved organic substances by means of their roots. The results of recent investigations have confirmed the theory. The phanerogamic plants studied have been found capable of absorbing and assimilating a considerable number of organic substances. The practical significance of this fact may be little, but, irrespective of the practical aspect, a thorough knowledge of the relation of the higher plants to dissolved organic substances is desirable and may be important in the interpretation of experimental data in other lines of research. Such a knowledge is desirable furthermore from the standpoint of a better understanding of metabolism in plants and because of its significance to animal physiology and biochemistry.

With a view to confirming and extending the investigations of previous workers, this study was begun in December, 1912, and the work here presented concludes that phase of the subject concerned with the absorption of certain carbohydrates and their influence on plant growth. As compared with previous investigations on this subject, a wider range and a larger number of plants have been employed; the study has been extended to the influence of weaker concentrations on growth; the continued influence of sugars on respiration has been studied; and an attempt has been made to obtain some data on the comparative ease of assimilability of different sugars. The last-named phase of the work is being more intensively studied in the light of the present investigation. Incidental observations have been made also on the influence of sugars on color production in the plants studied and on the excretion of enzymes, and the noteworthy fact developed that galactose is toxic to the plants and that its poisonous action can be antidoted. The prime object, however, has been to confirm the earlier work on the direct absorption of sugars, and to extend these studies to other plants.

HISTORICAL

De Saussure (1804) found that *Bidens cannabina* and *Polygonum persicaria* apparently absorbed sugar from an aqueous solution of the same. His work was of course not conducted under sterile conditions, and doubtless much of the sugar reported as being absorbed disappeared through fermentation. This work attracted no particular attention because of its agreement with the humus theory.

The possibility of absorption of organic substances by cuttings, leaves, and floral parts of plants was fully established by Boehm (1883), Meyer (1886), E. Laurent (1887), and Schimper and others (Pfeffer, 1900). The investigations of these men were designed to throw light on the question of the first product of photosynthesis. They found that starch was produced when the plant was offered any one of a number of organic substances, notably the sugars and glycerin.

After Boehm (1883) reported the absorption of sugar by means of the roots, Acton (1889) demonstrated the production of starch in deamylated plants at the expense of organic substances absorbed by the roots. He employed rooted plants of *Quercus robur*, *Cheiranthus cheiri*, *Euphorbia helioscopia*, *Phaseolus vulgaris*, and *Acer pseudoplatanus*. He noted that glucose, saccharose, and glycerin were absorbed and starch was produced in the leaves of the plants when they were maintained in liquid cultures in the dark through a period of a few days. Neither glycogen, dextrins, nor soluble starch were utilized. While the experiment was not conducted under sterile conditions, the conclusions need not be questioned because of the fact that the starch accumulated in the leaves when the plants were maintained in the dark and because the duration of the experiments was short.

Mazé (1899) grew vetch (vesces de Narbonne) under sterile conditions in the dark, supplying to the solution different quantities of glucose. Some of his data follow:

Duration (days)	Glucose (parts per 100)	Dry weight of plant (milligrams)	Dry weight of seed (milligrams)	Gain or loss (milligrams)
50	1	269.0	202.8	+ 66.2
39	2	276.7	202.8	+ 73.9
92	4	838.2	202.8	+635.4
92	6	710.0	202.8	+507.2
53	0	161.6	202.8	— 41.2

With 4 per cent and 6 per cent of glucose the plants grown in the dark more than trebled in dry weight, while in the absence of sugar there was a loss of weight.

The work of J. Laurent (1904) is particularly noteworthy. He grew plants in water cultures under sterile conditions. His conclusions, particularly with respect to the utilization of sugar by corn, are as follows: (1) With glucose present in the nutrient solution there is an increase in growth and an increase in dry weight. (2) Glucose is utilized if the plant is grown either under conditions of darkness or in the light, and in the absence of carbon dioxide as well as under normal conditions. Saccharose, glycerin, and potassium humate are also utilized. The saccharose is inverted in the root by the enzyme invertase. This sugar is also transformed in slight amounts in the culture solution, due to the secretion of invertase. The same is true for peas. Starch supplied to the nutrient solution is partially utilized, due to the exosmosis of diastase from the seed and its action on the supplied starch. (3) Glucose is absorbed by the roots of bean, ground ivy, wild marigold, and a number of other plants. The glucose gives rise to the formation of starch in the leaves of the plants studied.

There is also included in Laurent's paper a consideration of the effect of organic substances on the morphology and anatomy of peas.

Mazé and Perrier (1904), employing water cultures, grew corn under sterile conditions, noting the influence of saccharose, glucose, ethyl alcohol, and methyl alcohol on the growth. The plants grown in the dark for a period of forty-nine days had a dry weight of approximately fifty per cent of the original dry weight of the seed. Under the same conditions, except for the addition of saccharose, the plants showed a greater dry weight than that of the original seed. In the light the plants made excellent growth. In one culture of thirty days duration, the plant attained a dry weight of 21.9 grams, having absorbed in this time 10.446 grams of saccharose. The original amount of sugar added was 32.088 grams to the three-liter flask. At the end of the experiment there remained only 1.359 grams of saccharose, but 21.328 grams invert sugar. Invertase apparently was secreted by the roots. The saccharose seemed to be utilized more efficiently than the glucose. Glycerin and ethyl alcohol, while probably slightly assimilated, yet were injurious at the concentrations employed. Methyl alcohol 4.5 parts per 100 permitted better growth than the check plant made, and apparently was assimilated.

In a subsequent paper Mazé (1911) states that corn is capable of absorbing starch and peptone. The starch was not transformed in the culture solution, and, contrary to his previous results, Mazé did not note any inversion of the saccharose. The absorption of colloidal organic substances as starch is not in agreement with the results of other investigators.

Molliard (1907) studied the influence of various sugars, and also glycerin, on the growth, morphology, and anatomy of the common garden radish (*Raphanus sativus*), of cress (*Nasturtium officinale*), of morning glory (*Ipomoea purpurea*), and of onion (*Allium cepa*). He employed various sugar concentrations ranging from two to fifteen per cent, and noted that in the case of the radish the sugars could be absorbed and assimilated. Various morphological modifications were induced by the different concentrations with radish. Glucose, fructose, saccharose, and maltose influenced the plant similarly. Enlarged roots were produced only when the culture medium contained between five and fifteen per cent of glucose.

It was noted also that in the presence of certain concentrations of glucose, starch was produced in the root, although normally starch is not produced in radish roots. The following figures, taken from Molliard's paper, show the influence of concentration on growth under the conditions of the experiment:

INFLUENCE OF GLUCOSE ON RADISH. DURATION OF EXPERIMENT, THREE MONTHS

Knop's solution +	Dry weight (milligrams)	Ratio of dry weight to fresh weight
No glucose	50	0.056
2 per cent glucose	105	0.089
5 per cent glucose	177	0.110
10 per cent glucose	220	0.132
15 per cent glucose	128	0.167

As a result of certain other experiments Molliard concluded that there exists an antagonism between absorption of sugar and assimilation of carbon dioxide. This will be considered later.

Lubimenko (1906 b) has noted the assimilation of various sugars by plantlets of *Pinus Pinea*, and as a result of his experiments he comes to the conclusion that the assimilation of sugar is dependent on a photochemical reaction.

Bokorny (1911) found that Spirogyra and Cladophora not only could absorb methyl alcohol, but could produce starch from it. His results indicate further than the scarlet runner bean and the pea can also utilize methyl alcohol, but not ethyl alcohol nor the higher alcohols. His experiments were not made under sterile conditions.

In a recent paper Ravin (1913) has presented the results of his very comprehensive investigation of the influence of certain organic acids and their acid and neutral salts on the growth of radish as well as on certain algæ and fungi. He has presented an excellent résumé of the literature relative to the use of organic acids, so that no further treatment is here necessary. The common garden radish (*Raphanus sativus*) was employed, and the plants were grown under sterile conditions in large culture tubes of 350 cubic centimeters capacity. As a substratum for growth, cotton, pumice, or sand was employed. To this was added the nutrient solution to be tested. The results, summarized in the following table, clearly indicate that radish can absorb and assimilate the organic acids, such as malic, tartaric, succinic, and citric, and their salts, and comparison is made with glucose:

GENERAL AVERAGES OF RESULTS OBTAINED FOR THE DIFFERENT GROUPS OF SUBSTANCES

Culture medium	Free atmosphere			Confined atmosphere		
	Green weight (milligrams)	Dry weight (milligrams)	Relative acidity of plant	Green weight (milligrams)	Dry weight (milligrams)	Relative acidity of plant
Knop (check)	749	36.3	1.0	166	11.7	1.5
Knop + $KHSO_4$ (acid salt)	811	41.0	1.1	250	16.9	1.5
Knop + K_2SO_4 (neutral salt)	793	42.0	1.0	247	17.0	1.4
Knop + glucose	1,035	54.9	1.3	306	30.5	1.8
Knop + free organic acids	945	52.5	1.5	426	30.5	2.2
Knop + acid potassium salts of organic acids	1,030	53.9	1.4	515	33.3	1.9
Knop + neutral potassium salts of organic acids	947	53.6	1.2	466	28.7	1.7

Palladine (1901), Palladine and Komleff (1902), and Maige and Nicolas (1910), have studied particularly the influence of various organic substances on the rate of respiration. Their investigations will be considered subsequently in this paper.

Not only do the phanerogams have the ability to absorb organic substances, but the same property has been shown by algæ, as has been demonstrated by Bokorny (1911), Ravin (1913), and others. Recently Servettaz (1913) has investigated the absorption and assimilation of organic substances by mosses. He finds that glucose and fructose are readily utilized, whereas the disaccharides saccharose, lactose, and maltose are of less value and are absorbed in the order given. Dextrin, starch, and gum arabic seem to be detrimental to moss plants. Peptone also is utilized. The observation was made that the moss in the presence of sugar tends to be chlorotic, a similar observation also being noted by Mazé and Perrier (1904) for corn grown with glucose added to the culture solution. The suggestion was made by these authors that there seems to be a disappearance of chlorophyll with reduction of its function, although no evidence has been produced to show loss of function.

Investigations on the rôle of organic nitrogenous substances have been made largely from the viewpoint of the value of the substance supplied as a source of nitrogen. Lutz (1898), employing cultures free from micro-organisms, succeeded in demonstrating that certain of the amines could serve as sources of nitrogen. Hutchinson and Miller (1911) have extended the investigations of Lutz to other plants with favorable results. These authors have presented a critical survey of the work performed, and make the following statement concerning the previous work:

> As regards organic compounds the great majority have given negative, if not uncertain results. More or less satisfactory evidence of assimilation has been obtained with the following compounds — methyl-, amyl- and allylamines, dimethylamine, acetamide, choline, betaine, leucine, urea, dicyano-diamide, aspartic acid, asparagine, glutamine, allantoin, uric acid, hippuric acid, tyrosine and humic acid. The gains of nitrogen have, however, generally been very small and in many cases negative results have been obtained by other investigators.

It seems probable that some of the organic nitrogenous substances can supply the needs of the plant, although Russell (1912) is of the opinion that such of these substances as are apparently utilized are split at the surface of the root, yielding ammonia as one product which supplies the nitrogen for the plant.

As already stated, investigations of the utilization of organic nitrogenous substances have been made largely from the viewpoint of the nitrogen relationships. It is obvious, however, that one cannot entirely dissociate the carbon relationships when experimenting with such substances. It is this viewpoint that has guided Lefèvre (1906) in his investigation of the utilization of amino acids in the complete absence of carbon dioxide. Lefèvre has apparently demonstrated that certain amino compounds can serve as sources of carbon. He found that *Lepidium sativum, Ocimum minimum,* and *Tropaeolum varians nanum,* cultivated in an artificial soil of sand and moss, when grown under a bell jar and supplied with air freed of carbon dioxide, apparently utilized as a source of carbon the amino acids supplied. He used a mixture of tyrosine, glycocoll, alanine, oxamide, and leucine. Plants grown under these conditions increase in height and in number and size of leaves. Check plants grown under conditions similar but with the elimination of amino acids from the soil failed to make any marked growth, and some died of starvation. In one experiment with *Lepidium sativum* twenty seedlings were grown under a bell jar in the absence of carbon dioxide but with the amino-acids mixture present. In ten days the increase in weight was from 44 to 120 milligrams. The check culture, in the absence of the amino acids, showed a gain of only 4 milligrams. In a similar experiment with Ocimum, the gain of the seedlings supplied with the amino acids was from 100 to 350 milligrams, while the check plants showed a gain of only 20 milligrams. Jost (1913) has suggested that the amino acids may be absorbed and broken down in the plant, yielding carbon dioxide, which is utilized by the plant in photosynthesis.

Grafe (1909) conducted somewhat similar experiments, employing *Phaseolus vulgaris*. He used the same mixture of amino acids as was used by Lefèvre, but grew his plants in water cultures. He obtained, in general, toxic effects on the roots of the culture plants grown in the presence of the amino acids. His results do not, however, disprove the conclusions of Lefèvre, since in the experiments of the latter the soil employed undoubtedly inhibited the toxicity of the amino-acids mixture.

From a cons deration of the above-mentioned investigations it becomes evident that a considerable number of organic substances can be utilized by green plants and that these substances are of a wide range of character. The investigation here reported is confined to the influence of certain of the carbohydrates on green plants. The experiments are not in all cases so clear-cut and decisive in their results as was hoped for, but the difficulties of experimentation and the limited supply of culture vessels made ideal results almost impossible of attainment. However, the results are in general conclusive.

METHOD OF INVESTIGATION

CULTURE VESSELS

Throughout the investigation the plants were grown in culture vessels plugged with cotton and maintained under sterile conditions. In certain experiments the culture vessels were large test tubes, 30 by 4 centimeters in size; in other experiments large glass cylinders were employed, 63 by 10 centimeters in size; while in still other experiments the culture vessels were of another form, as described later. The use of large tubes is shown in figure 1.

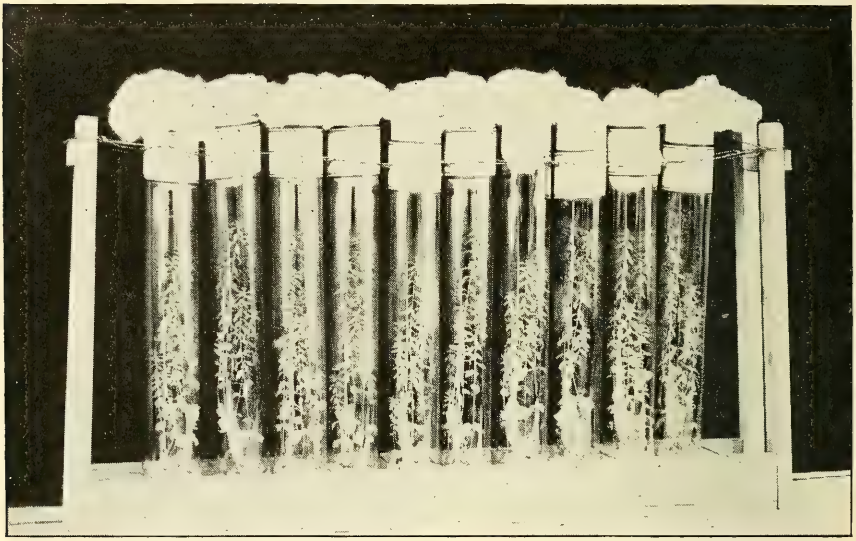

FIG. 1. METHOD OF GROWING PLANTS IN LARGE CULTURE TUBES

NUTRIENT SOLUTION

The plants were grown for the most part within the tubes or cylinders on an agar medium to which were added the nutrient solution and the carbohydrate whose influence was to be studied. Pfeffer's solution was employed, though of a weaker concentration than usual. The solution was made according to the following formula:

Calcium nitrate	4.0 grams
Potassium nitrate	1.0 gram
Potassium chloride	0.5 gram
Potassium phosphate (dibasic)	1.0 gram
Magnesium sulfate	1.0 gram
Ferric chloride	4.0 milligrams
Distilled water	12.0 liters

ACTION OF SOLUTION ON DISACCHARIDES

The inverting action of the nutrient solution on saccharose, maltose, and lactose was tested and found to be without effect. There was of course no hydrolyzing action on the agar.

STERILIZATION OF SEED

The method of seed sterilization employed was one developed in the Laboratory of Plant Physiology by Wilson (1915). In brief it is as follows: Ten grams of calcium hypochlorite (chloride of lime, or bleaching powder) is shaken up with 140 cubic centimeters of water, and after the mixture has stood for a short time the supernatant, clear liquid is decanted off and filtered. It is now ready for use. The seeds to be sterilized are placed in a test tube or similar vessel and the hypochlorite solution is added. The tube or vessel is tightly stoppered, and the seed is left in the solution for a few to twenty-four hours. The treatment of the various seeds will be described in detail later for each experiment.

STERILIZATION OF MEDIA AND CULTURE VESSELS

The culture vessels, containing the culture solutions, were sterilized under steam pressure. The smaller vessels were heated in an autoclave for twenty-five minutes at fifteen pounds pressure, while the larger containers were sterilized for one hour at ten pounds pressure in a large steam sterilizer such as is used in canning factories. Even at the prolonged exposure in the latter case, contamination of the saccharose cultures occasionally occurred, due to the development of submerged hyaline colonies of a bacterium. This organism occurred only in the saccharose cultures. Its appearance was evident five or six days after sterilization. All cultures showing the contamination were rejected.

SOWING THE SEED

For sowing the seed in certain of the experiments a small copper spoon was used. This was made of a small strip of copper, which was worked into a perforated spoon large enough to hold two vetch seeds and fitted with a wooden handle. The spoon was sterilized by immersion in alcohol and then flaming. The seed was transferred directly from the bleaching-powder solution to the culture vessels much as one might make transfers of bacteria or fungi. In many of the experiments each lot of seed was sterilized separately in small test tubes, each test tube holding the seeds for a single culture. This method was necessary when the weight of the seed was to be accurately determined.

UNIFORMITY OF SEEDS

In all the experiments the seeds used were carefully selected as regards uniformity in appearance and size. Only plump, vigorous-looking seeds were selected. In work of this character it is essential to use only seed of high germinating ability. The contaminations that occurred were due largely to the introduction of dead seeds.

RATE OF DIFFUSION OF CARBON DIOXIDE THROUGH COTTON PLUGS

Molliard (1907) made some experiments to find out whether or not the cotton plugs used in the culture tubes impeded the diffusion of carbon dioxide into the plant chamber. He placed in the plant chamber small open vessels containing baryta water. He found that the cotton plugs impeded the diffusion of carbon dioxide, since the baryta water in the plugged chambers absorbed only about one-fourth of the carbon dioxide absorbed by the baryta water in the open chambers.

In an attempt to verify Molliard's results the writer placed wide-mouthed bottles containing baryta water in the large cylinders, two of which were open and two closed. No good results were obtained, since the carbonate precipitate which formed at the surface interfered with the absorption of the carbon dioxide. Consequently the baryta water was replaced by a solution of potassium hydroxide of a specific gravity of 1.27. After a period of four days it was found that the potassium hydroxide in the plugged cylinder had absorbed only one-third as much carbon dioxide as that in the open cylinder. The cotton plugs, therefore, do impede the diffusion of carbon dioxide, and consequently for all this work it was essential to observe precaution in providing plugs of uniform compactness.

INFLUENCE OF CERTAIN SUGARS ON GROWTH OF CORN (ZEA MAYS L.)

J. Laurent (1904), and Mazé and Perrier (1904), investigated the absorption of sugar by corn. Their results, while clearly establishing the utilization of various sugars, do not give any idea of their respective assimilability. Mazé and Perrier state that saccharose is of more value than glucose, but the evidence is not conclusive, since in the presence of glucose abnormal plants developed in two cases and a normal plant in the third case. In the dark the plants seemed to utilize saccharose, lactose, glycerin, and starch in the order named.

In the following experiments the influence of the hexoses glucose and fructose, and the disaccharides saccharose and maltose, was noted. In one experiment the plants were grown in the laboratory, being maintained in a dark chamber, while in the second the plants were grown in the greenhouse. The assimilability of the sugar was judged by noting its influence on the dry weight of the plants. The variety of corn used was a yellow dent. All the grains were selected for uniformity of shape and weight, and all came from the same ear.

As culture vessels the large cylinders, 63 by 10 centimeters in size, were used, and in each was placed the nutrient solution plus 1.2 per cent of agar. Two-per-cent concentrations of the various sugars were used. These solutions are approximately equivalent as regards carbon, but differ as regards their osmotic relationships. The hexose sugars would have the higher osmotic pressure, since a 2-per-cent solution has a gram molecular concentration of 0.111 while the molecular concentration of the 2-per-cent disaccharide solutions is 0.058. Subsequent experiments and the work of Molliard (1907) indicate that concentrations of sugar much higher than that of the hexose cultures are more favorable to the growth of plants than the weaker concentration. In fact, in an experiment subsequently reported, 6.4 per cent of glucose shows a more favorable influence than 2 per cent of glucose. The higher molecular concentration of glucose as compared to saccharose, then, should not be injurious to the growth of corn. On the other hand, this increased concentration might account for the greater value of the hexose sugars in increasing growth.

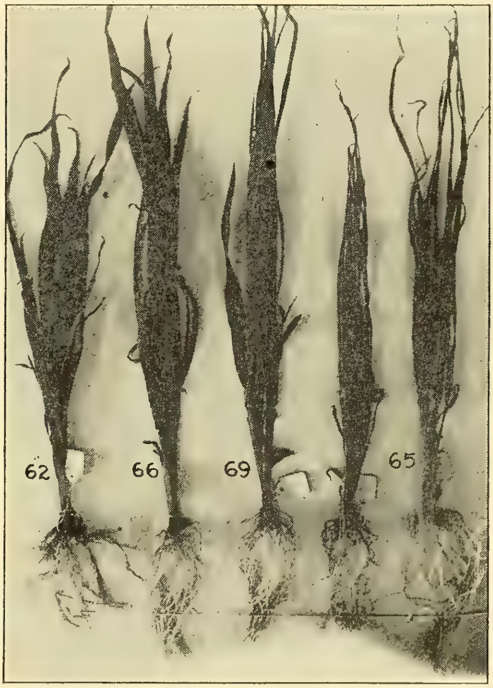

FIG. 2. INFLUENCE OF SUGARS ON GROWTH OF CORN

62, Glucose; 66, fructose; 69, saccharose; unnumbered, check (no sugar); 65, maltose

IN THE LIGHT

The results obtained from the cultures grown in the light are given in table 1. The results for each of the different sugars are consistent, though there is a slight difference in the individual cultures of each sugar in the distribution of growth. A very notable increase in dry weight is manifest in all the sugar-fed plants. Indeed, when supplied with sugar the dry weight of the plants is in most cases approximately double that of the check plants. Glucose appears to be the most effective, followed by fructose, saccharose, and maltose in the order given. No effect was noted of the sugars on the use of the endosperm reserve. Unfortunately the dry weight was obtained from only one of the check cultures, the plants of the other check being accidentally lost. The general appearance of the latter was very similar to the first check culture, as is indicated by the figures for average length of tops. The influence of the sugars is seen in figure 2.

TABLE 1. INFLUENCE OF VARIOUS SUGARS ON GROWTH OF CORN

(Plants grown in greenhouse. Duration, December 20 to January 19, 1914, thirty days. Original weight of seed, 0.296 gram ± 10 milligrams)

Culture solution		Number of plants	Average length of tops (centimeters)	Dry weight of tops (grams)	Dry weight of roots (grams)	Total dry weight (grams)	Average weight per plant (grams)
Check (no sugar)	1	4	39.5	......	......	......	....
	2	4	39.0	0.535	0.343	0.878	0.219
Glucose, 2 per cent	1	4	40.2	0.989	0.773	1.762	0.440
	2	4	38.0	0.958	0.735	1.693	0.423
Fructose, 2 per cent	1	4	49.0	1.078	0.672	1.750	0.437
	2	4	53.5	1.005	0.695	1.700	0.425
Maltose, 2 per cent	1	4	46.0	0.794	0.479	1.273	0.318
	2	4	52.0	0.802	0.444	1.246	0.311
Saccharose, 2 per cent	1	4	51.5	0.976	0.531	1.507	0.377
	2	4	47.0	0.830	0.695	1.525	0.381

IN THE DARK

The data for the plants grown in the laboratory in the dark are given in table 2. In all cases the average length of the tops is greater in the sugar-containing cultures than in the check cultures. The same is true of the dry weights; in the presence of sugar the plants not only made considerable growth, but approximately maintained their original dry weight.

No very appreciable differences were obtained with the different sugars; apparently the glucose was slightly the most beneficial.

TABLE 2. INFLUENCE OF VARIOUS SUGARS ON GROWTH OF CORN

(Plants grown in the dark. Duration, December 20 to January 19, 1914, thirty days. Original weight of seed, 0.296 gram ± 10 milligrams)

Culture solution		Number of plants	Average length of tops (centimeters)	Dry weight of tops (grams)	Dry weight of roots (grams)	Total dry weight (grams)	Average weight per plant (grams)
Check (no sugar)	1	4	50.7	0.400	0.180	0.580	0.145
	2	4	47.5	0.400	0.260	0.660	0.165
	3	4	50.5	0.440	0.210	0.650	0.162
Glucose, 2 per cent	1	4	61.5	0.650	0.580	1.230	0.307
	2	4	69.0	0.735	0.370	1.105	0.276
Fructose, 2 per cent		4	58.7	0.580	0.450	1.030	0.257
Maltose, 2 per cent	1	3	67.0	0.450	0.370	0.820	0.273
	2	4	64.0	0.715	0.310	1.025	0.256
Saccharose, 2 per cent		4	58.0	0.580	0.410	0.990	0.247

It is stated by Lindet (1911) that fructose is a tissue builder, while glucose is used largely in respiration. It would be expected, therefore, that fructose, especially when offered to plants grown in the dark, would effect a greater increase in growth than would glucose, and that saccharose would behave similarly. It may be, however, that glucose is more permeable, or that the statement does not hold for higher plants. There is nevertheless a possibility that further work might show such a relation.

INFLUENCE OF MALTOSE ON GROWTH OF CORN EMBRYO

In the experiment to determine the influence of maltose on the growth of the corn embryo, the embryo was sown instead of the entire seed. The embryo was first removed from the endosperm and all adhering starch was removed. It was then sterilized by immersion in the calcium hypochlorite solution for a period of four hours. As culture vessels test tubes 30 by 4 centimeters in size were employed, each containing 50 cubic centimeters of the nutrient solution plus maltose sugar at certain concentrations. The plants were grown in the greenhouse from January 3 to February 6, 1914, a period of thirty-four days. The check culture yielded a dry weight of 106 milligrams, the culture containing 0.4 per cent of maltose 130 milligrams, and the culture containing 2.5 per cent of maltose 180 milligrams. The utilization of maltose was made evident also by the very marked red coloration of the roots and stem of the plant in the culture containing 2.5 per cent of maltose, the fainter coloration of the plant in the culture containing 0.4 per cent of maltose, and the absence of color in the check cultures.

EXPERIMENTS WITH CANADA FIELD PEA (PISUM SATIVUM L.)

In the experiment with Canada field peas large cylinders, 46 by 8 centimeters in size, were used, each containing 150 cubic centimeters of the nutrient solution plus 1.2 per cent of agar and the sugar to be tested. The seeds were selected for uniformity, and each lot of four seeds was weighed and sterilized separately in small weighing dishes 15 cubic centimeters in volume. The duration of growth was thirty-five days. The sugars used and the results obtained are given in table 3:

TABLE 3. INFLUENCE OF VARIOUS SUGARS ON CANADA FIELD PEA

(Duration, thirty-five days)

Culture solution		Original dry weight of seeds (grams)	Dry weight			Total dry weight (grams)	Gain or loss (grams)
			Tops (grams)	Roots (grams)	Cotyledons (grams)		
Check (no sugar)	1	0.617	0.272	0.114	0.079	0.465	—0.152
	2	0.555	0.227	0.093	0.093	0.413	—0.142
	3	0.563	0.212	0.060	0.160	0.432	—0.131
Lactose, 2 per cent	1	0.585	0.246	0.105	0.150	0.501	—0.084
	2	0.567	0.291	0.120	0.108	0.519	—0.048
Maltose, 2 per cent	1	0.567	0.354	0.141	0.096	0.591	+0.024
	2	0.594	0.340	0.139	0.105	0.584	—0.010
	3	......	0.355	0.126	0.094	0.575	....
Glucose, 2 per cent	1	0.555	0.284	0.119	0.151	0.554	—0.001
	2	0.554	0.296	0.113	0.152	0.561	+0.007
Saccharose, 2 per cent	1	0.585	0.374	0.246	0.095	0.715	+0.130
	2	0.576	0.346	0.230	0.125	0.701	+0.125

Here again it is noted that all the sugars exert a beneficial effect on the plants. In this case saccharose is the most beneficial, glucose and maltose are somewhat similar, and lactose is of the least benefit. No conclusions can be drawn with respect to the conservation of the reserve food of the cotyledons because of the sugar supplied.

EXPERIMENTS WITH TIMOTHY (PHLEUM PRATENSE L.)

A considerable number of experiments were made with timothy, but most of the data are not included for the reason that the results obtained were not concordant. This was due to the fact that a constant number of plants could not be maintained in the culture vessels, owing to the difficulty of introducing a uniform number of seeds and to variation in germination. Various tests showed that there is a relation between the number of plants in the culture vessel, and growth of the plants. The greater the number of plants in the culture vessel, the less is the growth of the individual plants therein. Experiments indicate that the limiting factor is a supply of carbon dioxide.

In the following experiments with timothy the plants were grown in the large test tubes. In each tube was placed exactly 50 cubic centimeters of the culture solution containing 1.2 per cent of agar. The seeds were sterilized in the usual manner. The duration of growth varied in the different experiments.

CULTURES IN LIGHT

In this experiment the influence of 2-per-cent solutions of glucose, maltose, and saccharose was to be noted. Unfortunately the maltose and the saccharose cultures became contaminated. Likewise unfortunately for accurate results, the same number of plants was not present in each culture. Since, however, there are fewer plants in the check than in the glucose cultures, the beneficial effect of glucose is in reality greater than the dry-weight figures indicate. The plants grown in the presence of glucose showed a more vigorous appearance than did the check plants. Also, the plants in the presence of glucose showed at the base of the stalk a markedly red coloration, which disappeared after the plants had been kept in the laboratory for a week. The results obtained are given in table 4. In another experiment it was noted that asparagin could serve timothy as a source of nitrogen.

TABLE 4. INFLUENCE OF GLUCOSE ON TIMOTHY

(Plants grown in greenhouse. Duration, April 5 to May 27, 1913, fifty-two days)

Culture solution		Number of plants	Total dry weight (milligrams)	Average weight per plant (milligrams)	Notes
Check no (sugar)	1	17	41	1.11	
	2	15			
	3	5			
Glucose, 2 per cent	1	13	70	1.63	Plants reddish at base
	2	13			
	3	17			

CULTURES IN DARK

In the experiment to determine the influence of sugars in the dark, the plants were grown in the same manner as in the preceding experiment. The solutions used are indicated in table 5. All conditions of the experi-

TABLE 5. INFLUENCE OF VARIOUS SUGARS ON TIMOTHY GROWN IN INCUBATOR AT 32° C.

(Duration, fifty-two days)

Culture solution		Number of plants	Average height of tops (centimeters)	Total dry weight (milligrams)	Average dry weight per plant (milligrams)
Check (no sugar)	1	6	2.30	5	0.11
	2	29	2.02		
	3	10	2.00		
Glucose, 2 per cent	1	27	3.95	16	0.28
	2	15	5.26		
	3	14	5.85		
Maltose, 2 per cent	1	9	3.88	10	0.24
	2	10	3.45		
	3	22	3.93		
Lactose, 2 per cent	1	18	3.44	12	0.24
	2	16	3.93		
	3	15	3.96		

ment were the same as in the preceding, except that the cultures were kept in the dark in an incubator at a temperature of 32° C. In this experiment the number of plants again differed in the various cultures, but it was found that under the condition of growth — namely, darkness — it is practically immaterial whether or not the number of plants is the same in each culture. When the plants are grown in the light, the greater the number of plants the less is the opportunity for their securing carbon dioxide. In darkness, however, the growth is at the expense of the reserve food or sugar supplied, and the available food under the conditions of the experiment is the only limiting factor.

The results are presented in table 5. It will be noted that glucose, lactose, and maltose are all better than the check. The average height of the plants is least in the check and greatest in the glucose. The difference in the vigor of the different cultures was plainly visible. It is interesting to note that here lactose is apparently utilized, while in other experiments, when the plants were grown in the light, this sugar was not utilized.

INFLUENCE OF LACTOSE

In a preliminary experiment with timothy it was noted that no increase in growth over the check plants was obtained when lactose was supplied

TABLE 6. Influence of Various Concentrations of Lactose on Growth of Timothy

(Plants grown in greenhouse. Duration, December 18 to February 18, 1914, sixty-two days)

Culture solution		Number of plants	Total dry weight (milligrams)	Average dry weight per plant (milligrams)
Check (no sugar)	1	60	79.4	1.32
	2	59	63.0	1.07
	3	64	84.0	1.31
Lactose, 0.1 per cent		65	69.0	1.06
Lactose, 0.8 per cent		60	73.0	1.22
Lactose, 1.0 per cent		65	85.0	1.31
Lactose, 2.0 per cent	1	52	66.0	1.27
	2	68	76.0	1.12
Lactose, 2.5 per cent		57	74.0	1.30

to plants grown in the light. In the following experiment a series of various concentrations of lactose were tested, with results as shown in table 6. A large number of seeds were sown in each culture tube, and in no case was there increased growth. Lactose is therefore not assimilated by timothy when the plant is grown in the light.

INFLUENCE OF A MIXTURE OF LACTOSE AND SACCHAROSE

In the preceding experiment it was noted that lactose exercises no beneficial effect on timothy. In the following experiment a mixture of lactose and saccharose was employed. With 0.4 per cent of saccharose added to 2 per cent of lactose there is a marked increase in growth, and still greater growth results when 0.8 per cent of saccharose is added to 2 per cent of lactose. It appears, therefore, that there is an election of saccharose by timothy, and that this sugar is directly assimilated by the plant. The results are given in table 7:

TABLE 7. INFLUENCE OF A MIXTURE OF LACTOSE AND SACCHAROSE ON GROWTH OF TIMOTHY

(Duration, thirty days)

Culture solution		Number of plants	Total dry weight (milligrams)	Average dry weight per plant (milligrams)
Check (no sugar)	1	60	79	1.32
	2	59	63	1.07
	3	64	84	1.31
Lactose, 2 per cent	1	52	66	1.27
	2	68	76	1.12
Lactose, 2 per cent, + saccharose, 0.4 per cent		60	85	1.42
Lactose, 2 per cent, + saccharose, 0.8 per cent		68	124	1.82

CONCLUSION

The results here reported, and the general results of three other extensive experiments with timothy not here reported, demonstrate that the sugars glucose, maltose, and saccharose are absorbed and assimilated by timothy. Lactose is apparently not utilized at all.

EXPERIMENTS WITH RADISH (RAPHANUS SATIVUS L.)

Radish has been used in experiments by Molliard (1907) and by Ravin (1913). Molliard concluded that radish is able to utilize glucose somewhat better than fructose. Maltose is much like glucose in its effect. The following figures from Molliard show the effect of various culture solutions on dry weight of radish:

Culture solution	Dry weight (milligrams)
Knop solution alone	42– 60
Knop + 5 per cent of glucose	109–133
Knop + 5 per cent of saccharose	102–125

Molliard states also that 9.5 per cent of maltose had the same effect as 5 per cent of glucose, with which it is isotonic. The work of Ravin on the influence of glucose and organic acids on radish has already been mentioned.

INFLUENCE OF APPROXIMATELY CARBON-EQUIVALENT SOLUTIONS

The variety of radish used in these experiments was the common garden variety. The seeds were carefully selected for uniformity of size. As culture vessels liter flasks were employed, each containing 200 cubic centimeters of the culture medium (Fig. 3). The seeds were sterilized by immersion in the calcium hypochlorite solution for a period of five hours. Fourteen cultures were set up for each series, and in each flask two seeds were sown. Not all the seeds germinated. In some cultures two plants, and in others only one plant, developed. The latter condition was particularly true for the check series of cultures; it may be that the sugar in the nutrient solution increased the percentage of germination. Because of the difference in germination the final results are given in two tables, since, as has been heretofore stated, a just comparison cannot be made between cultures having unequal numbers of plants. The results appear in tables 8 and 9:

TABLE 8. INFLUENCE OF VARIOUS SUGARS ON GROWTH OF RADISH
(Two plants in each culture. Duration, February 5 to April 17, 1914, seventy-one days)

Dry weight (in grams)				
Check (no sugar)	Saccharose, 2 per cent	Maltose, 2 per cent	Glucose, 2 per cent	Lactose, 2 per cent
0.960	1.200	1.250	2.250	1.350
0.620	0.950	1.200	0.850	1.050
0.605	1.900	3.060	2.200	1.900
0.775	0.900	0.900	1.150	0.500
0.835		2.400	1.760	1.200
0.950		0.700	1.820	0.700
		1.000	1.220	1.520
		1.150	2.170	0.900
		1.500	1.520	1.030
		2.500	1.720	0.900
		2.000	1.720	1.330
		1.200	2.670	0.730
			1.910	1.380
			1.160	1.300
			2.170	
			1.140	
			2.400	
			1.000	
Average 0.791	Average 1.237	Average 1.572	Average 1.713	Average 1.128

All the sugar cultures, with the exception of those containing 2 per cent of glucose plus 2 per cent of fructose produced better growth than did the check cultures having no sugar. Comparing the glucose cultures with the check cultures it is noted that the dry weight of the former is more than double that of the latter. Maltose and glucose are somewhat similar in their effect. Lactose also is evidently absorbed and assimilated by the plant. Molliard (1907) also reported the use of lactose, although according to him it was utilized only slightly by the radish.

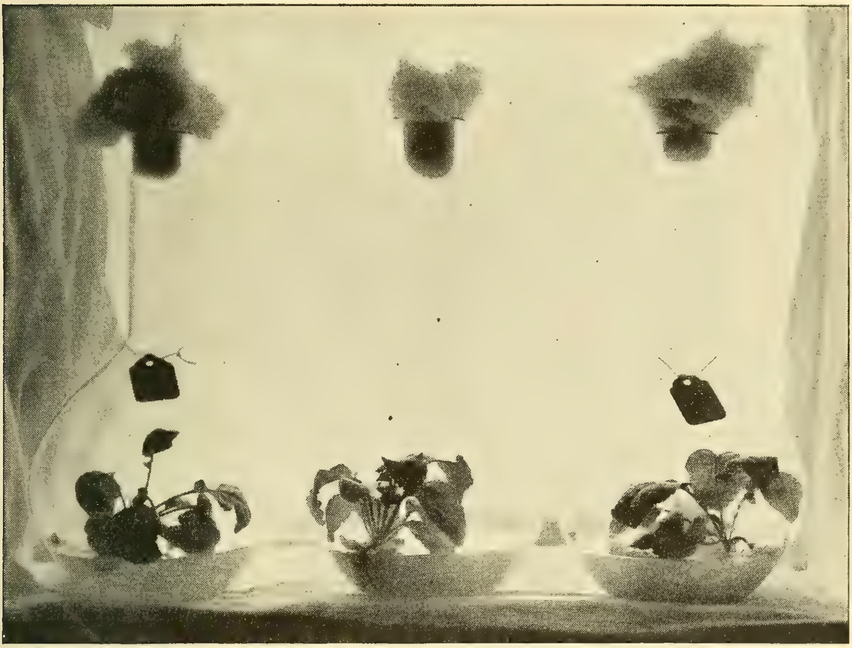

FIG. 3. METHOD OF GROWING RADISH

TABLE 9. INFLUENCE OF VARIOUS SUGARS ON GROWTH OF RADISH
(One plant in each culture. Duration, seventy-one days)

Dry weight (in grams)				
Check (no sugar)	Maltose, 2 per cent	Glucose, 2 per cent	Lactose, 2 per cent	Glucose, 2 per cent, fructose, 2 per cent
1.120	1.600	2.460	2.400	2.040
1.000	2.800	2.130	1.690	1.350
1.770	1.450	2.370	1.630	1.040
0.810	....	2.620	1.880	1.100
1.420	....	2.890	....	1.400
1.215	....	2.000	....	1.420
1.050	....	2.210	....	....
1.150	....	3.010	....	....
0.900	....	....	....	....
1.650	....	....	....	....
Average 1.208	Average 1.950	Average 2.461	Average 1.900	Average 1.392

In each culture having two plants one of the plants was usually larger than the other. It is not possible, therefore, to select individual plants as a criterion of the influence of the sugar. The total yield of each culture, or the average yield, affords the only means of comparison.

The influence of saccharose on the growth of radish is shown in table 10:

TABLE 10. INFLUENCE OF SACCHAROSE ON GROWTH OF RADISH
(Duration, February 16 to April 17, 1914, sixty days)

Dry weight (in grams)	
Check	Saccharose, 2 per cent
0.900	1.100
0.800	1.100
0.800	1.555
0.570	0.600
0.870	1.300
0.400	1.100
1.206	2.200
0.470	0.350
....	1.240
....	0.720
....	1.320
....	0.750
....	1.200
....	0.600
Average 0.752	Average 1.081

INFLUENCE OF EQUIMOLECULAR SOLUTIONS OF VARIOUS SUGARS

In the preceding experiments approximately carbon-equivalent solutions were employed. In the following experiment isotonic solutions were used, the concentrations of the various solutions tested being 0.05 molecular. Liter flasks were employed as culture vessels, each containing 200 cubic centimeters of the nutrient solution plus 0.5 per cent of agar.

The variety of radish was the same as that used in the preceding experiment, the seeds varying between 12 and 14 milligrams in weight. Only one seed was sown in each flask. The experiment was begun on February 8 and closed on April 15, the duration being sixty-six days. The results are given in table 11:

TABLE 11. INFLUENCE OF EQUIMOLECULAR SOLUTIONS OF 0.05 MOLECULAR CONCENTRATION ON GROWTH OF RADISH

(Duration, sixty-six days)

Culture solution		Dry weight (milligrams)	Sugar absorbed (milligrams)
Check (no sugar)	1	30	....
	2	35	....
Maltose	1	100	....
	2	175	....
	3	125	....
	4	140	....
Lactose	1	100	....
	2	75	....
Glucose	1	120	80.6
	2	162	75.6
Saccharose	1	195	105.0
	2	185	86.2
	3	145	....

It was hoped that the experiment would yield decisive results, for originally ten cultures were prepared for each sugar and fourteen for the check. Only a few of the seeds showed good germination, however, and consequently only thirteen of the original fifty cultures developed plants. The results nevertheless do show the very favorable influence of the sugars on growth. Lactose again is the least beneficial, yet it markedly increases the dry weight.

In none of these cultures was a storage root produced. In one saccharose culture not included in the table, which was badly contaminated with a species of Penicillium and a species of Fusarium, an enlarged root 2 centimeters long by 1 centimeter in diameter was produced. This production was probably due to the augmented carbon dioxide content of the flask, which increased the rate of photosynthesis and consequently produced a more nearly normal plant. In all the other cultures it is probable (see page 20) that the carbon dioxide content of the vessels was lower than the normal, due to the decreased rate of diffusion through the cotton stoppers.

Another interesting observation was made in one of the lactose cultures. The seed germinated, and the cotyledons increased in size to about 1 centimeter in diameter and then turned yellow. Increase in size continued until finally a root 1 centimeter in length and 0.5 centimeter in diameter was developed. It appears that in this case the radish behaved as a saprophyte.

In two of the maltose cultures not included in the table, the leaves, instead of being compound, were simple, narrow, and much elongated, which is suggestive of certain morphological modifications obtained by Molliard (1907).

EXPERIMENTS WITH WINTER VETCH (VICIA VILLOSA ROTH)

Winter vetch was selected for the more detailed studies of the influence of the different sugars on plant growth. This plant was chosen because of the ease of obtaining uniform seed, the ease of handling, the lack in the seed of a very large reserve, and the resistance of the seed to the sterilizing agent. Furthermore, the plant is one that grows rapidly, and in its early seedling stage is possessed of an anthocyanin pigment.

INFLUENCE OF CERTAIN DISACCHARIDES ON GROWTH

In the experiment to determine the influence of certain disaccharides on growth the large cylinders were used as culture chambers. Each cylinder contained 500 cubic centimeters of the nutrient solution plus 1.2 per cent of agar and the sugar to be tested. The seeds selected were uniform in character and of an average dry weight of 30 milligrams, the variation in weight being 2 milligrams. The seeds were sterilized by immersion in the calcium hypochlorite solution for nine hours. One series of cultures was maintained in the dark, and a second series was maintained in the greenhouse. The former cultures were grown in the laboratory at an average temperature of 23° C. The results of the experiment on cultures kept in the dark are summarized in table 12:

TABLE 12. INFLUENCE OF VARIOUS SUGARS ON GROWTH OF VETCH IN THE DARK
(Duration, fifty-four days. Dry weight of seed, 30 milligrams)

Culture solution		Length of tops (centimeters)		Length of roots (centimeters)		Total dry weight (milligrams)	Dry weight per plant (milligrams)	Loss in weight per plant (milligrams)
Check (no sugar)	1	12.5 19.0 15.0 20.0	16.6	2 3 3 3	2.7	67	16.7	13.3
	2	13.0 13.0 27.0	17.7	7 4 3	4.7	50	16.7	13.3
Lactose, 2 per cent	1	36.0 16.0 34.0	28.7	9 4 8	7.0	57	19.0	11.0
	2	25.0 25.0 29.0	26.3	9 8 5	7.3	60	20.0	10.0
Maltose, 2 per cent		26.0 28.0 21.0	25.0	9 6 5	6.7	65	21.7	8.3
Saccharose, 2 per cent		40.0 35.0 16.0 22.0 35.0	29.6	10 10 7 7 7	8.2	118	23.6	6.4

The check cultures grown in the dark lost nearly one-half of the original dry weight. The saccharose, on the other hand, permitted considerable growth involving a large expenditure of energy but at only a very small loss of the original weight. In every case better growth occurred in the presence of a sugar, the saccharose being better than the maltose, and the maltose better than the lactose, in the nutrition of the plant. In this connection it was noted that the agar medium containing saccharose showed reducing sugars in the immediate region of the roots, but none was detected in the central part of the agar mass. As stated previously, the nutrient solution is without effect on saccharose, and consequently the presence of reducing sugars must be ascribed to the secretion of the enzyme invertase or to the excretion from the roots of the reducing sugar.

The results obtained with cultures grown in the greenhouse are given in table 13:

TABLE 13. INFLUENCE OF VARIOUS SUGARS ON GROWTH OF VETCH IN THE GREENHOUSE
(Duration, sixty-three days. Weight of seed, 30 milligrams)

Culture solution		Culture number	Number of plants	Total dry weight (milligrams)	Average dry weight per plant (milligrams)	Gain in weight per plant (milligrams)
Check (no sugar)	1	73	5	348	70	40
	2	83	4	298	74	44
	3	85	2	157	78	48
Saccharose, 2 per cent	1	71	3	499	166	136
	2	81	4	646	161	131
Maltose, 2 per cent	1	70	4	477	119	89
	2	84	4	464	116	86
	3	74	4	406	101	71
Lactose, 2 per cent		82	3	300	100	70

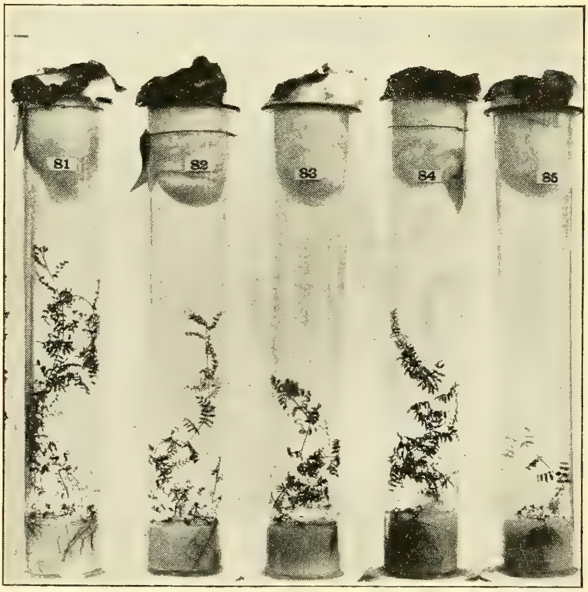

FIG. 4. INFLUENCE OF SUGAR ON VETCH
81, Saccharose; 82, lactose; 83, check (no sugar); 84, maltose; 85, check (no sugar)

Notes were taken on the lengths of roots and tops, but the root length and the height of tops varied with the degree of branching. Consequently the figures obtained are not indicative of the vigor of the plants. The differences in the cultures were easily observable, as is shown by representative cultures in figure 4.

All the plants were tested for starch and also for reducing sugars. No starch was found in any of the plants, but in those grown in the presence of saccharose traces of reducing sugars were detected by the use of Benedict's (1908–09) solution. The agar in the immediate region of the traversing roots showed traces of reducing sugars, which were made more evident by the use of Benedict's solution. The agar from the center of the mass through which the roots had not penetrated gave no indication of reducing sugars. The agar was also tested for hexose sugar by means of osazone tests. None was noted except in the saccharose culture.

A number of the cultures of this series were examined on March 7, and the results as regards green weight determinations at that time are given in table 14. The results are concordant with the data obtained from the cultures examined on March 16 (table 13). Unfortunately the saccharose culture examined on March 7 showed contamination by a yeast, and its increased growth may have been due in part to an increased supply of carbon dioxide produced as a result of this contamination.

TABLE 14. INFLUENCE OF VARIOUS SUGARS ON VETCH GROWN IN THE GREENHOUSE
(Duration, fifty-four days)

Culture solution	Length of tops (centimeters)	Length of roots (centimeters)	Total green weight (grams)	Green weight per plant (grams)
Check (no sugar)	34.0	17.1	1.629	0.407
Maltose, 2 per cent	37.0	15.0	3.720	0.930
Lactose, 2 per cent	27.5	21.0	2.386	0.596
Saccharose, 2 per cent *	47.0	26.0	6.428	1.607

* This culture was contaminated by a yeast.

INFLUENCE OF VARIOUS SUGARS ON VETCH GROWN IN WATER CULTURES

In all the preceding experiments agar media were employed. The roots of plants do not readily penetrate the agar, and furthermore the adsorptive properties of the agar probably interfere somewhat with the absorption of the dissolved substances, as well as lessen the action of any enzymes that might be secreted by the roots. For these reasons, and also when it is desired to measure quantitatively the amount of sugar absorbed by the plants in the various cultures, the use of water cultures is advantageous.

In the experiments with water cultures museum cylinders 30 by 50 centimeters in size were used as culture vessels, each containing 50 cubic centimeters of the nutrient solution to be tested. As a support for the seedling each cylinder was provided with a perforated aluminum receptacle (the lower part of a tea ball, the holes of which had been greatly enlarged) suspended by a string just below the cotton plug with which the cylinder was fitted. The whole was then sterilized in the autoclave. It was necessary to immerse the base of the cylinder in a pan of water in order to avoid breakage during sterilization. Before sowing the seed the part of the string on the outside was sterilized by moistening with calcium hypochlorite solution and wiping with a piece of cotton saturated with alcohol. The cotton plug and the string were then flamed. Moistening the string with the calcium hypochlorite solution removed all danger of its burning, and also aided in the sterilization of the string. Two seeds were planted in each receptacle, and the receptacles were then lowered so as to be just

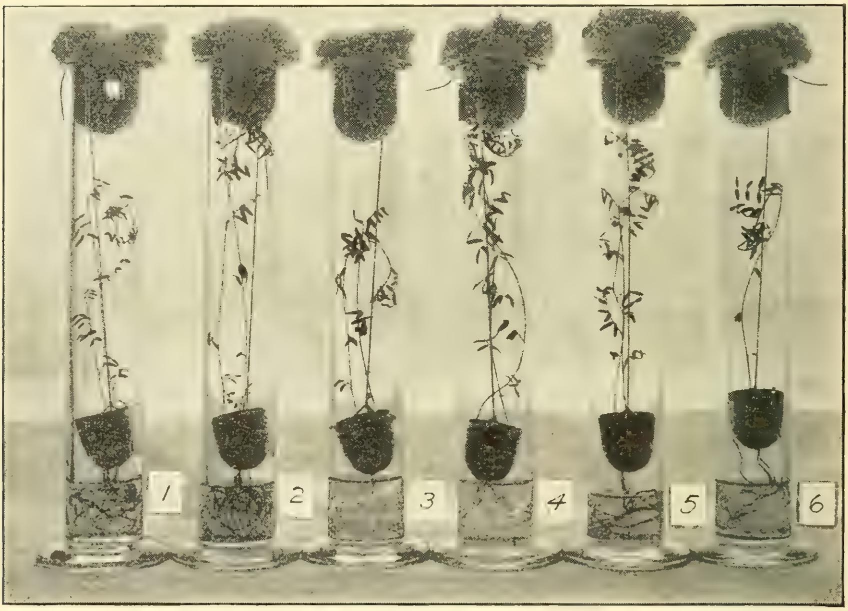

FIG. 5. INFLUENCE OF SUGAR ON VETCH. WATER CULTURE
1, Saccharose; 2, fructose; 3, lactose; 4, check (no sugar); 5, glucose; 6, maltose

above the surface of the nutrient solution (Fig. 5). The seeds were selected for uniformity of shape and size, each having a dry weight of 29 milligrams. They were sterilized in the usual way and two seeds were sown in each cylinder. The cultures were made in quadruplicate. The concentration of sugar employed was in all cases approximately 1 per cent. Owing to delayed germination of some of the seeds, to failure of germination of others, and to contamination in a few cultures, only one-half of the cultures were available for comparison. The sugars were determined by the Ahllin modification of the Fehling method. The disaccharide sugars were hydrolyzed and the reducing values were then determined. The results obtained are presented in table 15:

TABLE 15. INFLUENCE OF VARIOUS SUGARS ON THE GROWTH OF VETCH IN WATER CULTURES

(Duration, January 19 to February 27, 1915, thirty-nine days)

Culture solution		Number of plants	Dry weight of roots (milligrams)	Dry weight of tops (milligrams)	Total dry weight (milligrams)	Gain in weight (milligrams)	Average dry weight per plant (milligrams)	Gain in weight per plant (milligrams)	Sugar absorbed (milligrams)
Check (no sugar)	1	2	13	67	80	22	40.0	11.0	......
	2	2	15	68	83	25	41.5	12.5	......
	3	1	8	32	40	11	40.0	11.0	......
Lactose		1	16	40	56	27	56.0	27.0	49.0
Glucose	1	2	41	81	122	64	61.0	32.0	72.6
	2	1	30	62	92	63	92.0	63.0	66.5
Maltose		1	20	42	62	33	62.0	33.0	24.4
Fructose		2	39	75	114	56	57.0	28.0	113.0
Saccharose	*1	1	55	60	115	86	115.0	86.0	133.4
	†2	2	77	96	173	115	86.5	57.5	141.0

* 151 milligrams of reducing sugar present.
† 125 milligrams of reducing sugar present.

In every case there is a marked increase in yield when sugar has been supplied. The results are not conclusive, however, with respect to the order of assimilability, although saccharose again appears to be the most favorable. In all cases the amount of sugar utilized is greater than the dry weight increase except in the maltose culture. This is in keeping with the results of J. Laurent (1904), and is explainable by the high rate of respiration when the plant is provided with sugar (Palladine and Komleff, 1902, and Maige and Nicolas, 1910).

The plants grown in fructose, glucose, saccharose, and maltose were easily distinguished from the check plants not only by a more vigorous growth but also by the development of pigment throughout the entire length of the stem. The color varied from pink to purplish, the fructose culture showing a darker color than the others. Little color was produced in the presence of lactose. The roots of the plants grown in the presence of saccharose or fructose were in general thicker and showed a tendency to cork formation. In general the roots of all the sugar culture plants except those grown in lactose were very much branched, while those of the check plants were long and slender (Fig. 5).

As in the preceding experiments, it was found that the saccharose of the culture medium was partially inverted. The reducing sugar determined in saccharose culture 1 was equivalent to 0.151 gram of glucose, while that in saccharose culture 2 was equivalent to 0.125 gram. Approximately one-fourth of the original sugar content was found inverted at the conclusion of the experiment. The check solutions did not give even a qualitative test for reducing sugar, and no transformation of the lactose or of the maltose was found to have occurred.

A second experiment, similar to the preceding, was set up, the object of which was a comparison of glucose and saccharose on the growth of vetch in water cultures. Again the museum jars were used as culture vessels, with perforated aluminum receptacles for supporting the plants. The concentration of sugar employed was 1 per cent, and 50 cubic centimeters of the solution was used in each culture. The experiment was begun on March 30, 1915, and concluded on April 24. Unfortunately the comparison of glucose and saccharose is not conclusive, since in each of the glucose cultures only one plant developed. However, if glucose 2 is compared with saccharose 2 the advantage is in favor of saccharose. The results are given in table 16:

TABLE 16. INFLUENCE OF GLUCOSE AND SACCHAROSE ON GROWTH OF VETCH IN WATER CULTURE

(Duration, twenty-five days. Weight of seed, 30 milligrams)

Culture solution		Number of plants	Dry weight of tops (milligrams)	Dry weight of roots (milligrams)	Total dry weight (milligrams)	Gain or loss in weight (milligrams)	Sugar absorbed (milligrams)
Check (no sugar)	1	2	62	9.0	71.0	+11.0	....
	2	1	25	3.5	28.5	— 1.5	....
Glucose..........	1	1	52	7.0	59.0	+29.0	39.0
	2	1	49	8.0	57.0	+27.0	36.0
Saccharose.....	*1	2	81	31.0	112.0	+52.0	85.7
	†2	2	80	50.0	130.0	+70.0	64.6
	‡3	1	60	16.0	76.0	+46.0	55.0

* 66 milligrams of reducing sugar present.
† 50 milligrams of reducing sugar present.
‡ 88 milligrams of reducing sugar present.

INFLUENCE OF EQUIMOLECULAR SOLUTIONS OF GLUCOSE, MALTOSE, AND SACCHAROSE

In all the preceding experiments in which the comparative effects of the different sugars were to be noted, the sugars were used in approximately carbon-equivalent solutions, the concentrations being 1 or 2 per cent. Saccharose seemed to be utilized more readily than glucose, and glucose more readily than maltose. Lactose, as was to be expected, proved of the least benefit. Since the carbon-equivalent solutions are not all equimolecular, the osmotic relations of the disaccharides and the hexoses are different. As already stated, the 2-per-cent glucose solution is equivalent to a 0.111 gram molecular solution, while the 2-per-cent disaccharide is equivalent to a 0.058 gram molecular solution. The osmotic pressure (theoretical) of the glucose solution is therefore approximately twice as great as that of the disaccharide, and, as compared with the disaccharide, glucose might be expected to act disadvantageously to the growth of the plants because of this higher osmotic pressure. The increased growth with increase in concentration above 2 per cent of glucose indicates, however, that the 2-per-cent glucose solution has an advantage over the saccharose.

In order to obviate this condition, equimolecular solutions of glucose, maltose, and saccharose were employed. While with the carbon-equivalent solutions glucose is probably at an advantage because of increased osmotic pressure, with equimolecular solutions glucose suffers by comparison because of less available carbon. For every molecule of saccharose entering the plant there is made available one molecule of glucose and one of fructose, by virtue of the action of the enzyme invertase. Thus it is evident that glucose would have to penetrate twice as rapidly as saccharose in order to produce the same effect.

In this experiment the glucose, maltose, and saccharose used were of 0.05 gram molecular concentration in Pfeffer's solution at one-half its normal strength. Large cylinders were used, containing 200 cubic centimeters of the nutrient solution plus 0.5 per cent of agar. In each cylinder two seeds, each weighing 32 milligrams, were sown, after being sterilized in the usual way. The cultures were made in quadruplicate, but again, as in the preceding experiment, a number of the cultures had to be discarded because of delayed germination and other conditions. The sugar determination was made gravimetrically by the Fehling method. The nutrient medium was made up to 1000 cubic centimeters, and the sample taken for analysis was freed of agar by precipitation with lead subacetate; the lead being removed by treatment with anhydrous sodium sulfate. The results obtained are given in table 17:

TABLE 17. INFLUENCE OF EQUIMOLECULAR SUGAR SOLUTIONS ON VETCH

(Duration, thirty-three days)

Culture solution	Number of plants	Green weight (grams)	Dry weight of roots (grams)	Dry weight of tops (grams)	Total dry weight (grams)	Gain in weight (grams)	Sugar at beginning of experiment (grams)	Sugar at end of experiment (grams)	Sugar absorbed (grams)
Check (no sugar)...... 1	2	1.82	0.036	0.133	0.169	0.105	......	......	....
2	2	1.52	0.028	0.128	0.156	0.092	......	......	....
Glucose...... 1	2	1.90	0.040	0.153	0.193	0.129	1.759	1.675	0.084
2	2	2.30	0.043	0.213	0.256	0.192	1.759	1.660	0.099
Maltose...... 1	1	1.32	0.027	0.113	0.140	0.108	3.577	3.545	0.032
2	2	2.02	0.054	0.150	0.204	0.140	3.577	3.480	0.097
Saccharose... *1	2	2.55	0.080	0.190	0.270	0.206	3.666	3.544	0.122
†2	2	2.40	0.073	0.190	0.263	0.199	3.666	3.550	0.116

* 0.302 gram of reducing sugar present.

† 0.342 gram of reducing sugar present.

Here again the results are not so consistent as might be expected. The only conclusion to be drawn is that sugar is absorbed and increases the growth of the plants. Saccharose again appears to be more beneficial than glucose or maltose. Further evidence is obtained relative to the secretion of invertase from the roots.

INFLUENCE OF GLUCOSE ON ROOT GROWTH

In every culture in which a sugar was used a very marked effect on root growth was noted. In one experiment vetch was grown for ninety days, on the one hand in 0.05 molecular concentration of glucose and on the other hand in the absence of glucose. The influence of the glucose on root growth is graphically illustrated in figure 6.

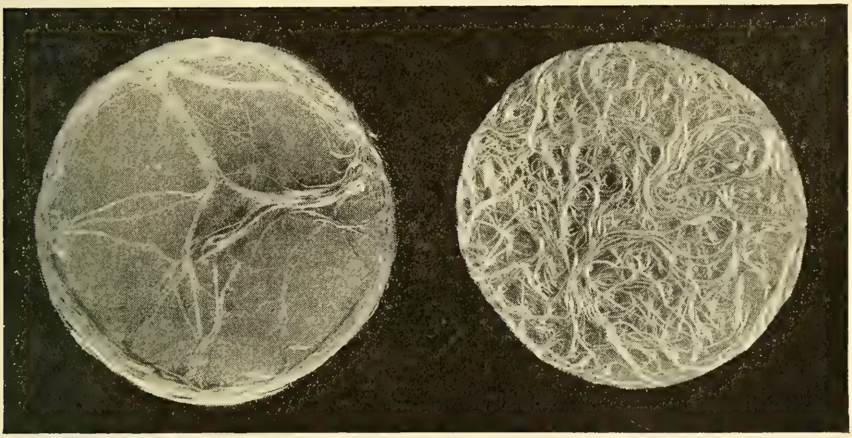

FIG. 6. INFLUENCE OF GLUCOSE ON GROWTH OF ROOTS OF VETCH
Left, check (no sugar); right, glucose culture
(Photographed through bottom of large glass cylinder)

INFLUENCE OF SUGAR ON RESPIRATION AND GROWTH

Palladine (1901) noted the influence of various sugars on the respiration of etiolated leaves of *Vicia Faba*. The leaves were left for a time in various sugar solutions and their respiration was then determined. The substances used, in the order of their accelerating effect on respiration, were fructose, glucose, saccharose, maltose, raffinose, glycerin, and mannite.

Maige and Nicolas (1910) studied the influence of various concentrations of different sugars on the turgescence and respiration of etiolated shoots of bean and the excised embryo of kidney bean. The plant material was immersed in the sugar solutions and the respiratory rate was then measured. The experiments were not made under sterile conditions, but since the duration of the respiration determination was but one hour this precaution was not necessary. With the etiolated shoots of bean the following data were obtained on the relative increases in evolution of carbon dioxide, the intake of oxygen, and the respiratory coefficients. The concentration of sugar was 10 parts per 100.

	Carbon dioxide (CO_2) (grams per hour)	Oxygen (O_2) (grams per hour)	$\frac{CO_2}{O_2}$
Saccharose	1.73	1.28	1.35
Maltose	1.47	1.26	1.16
Lactose	1.05	0.95	1.09
Glucose	1.75	1.32	1.32
Fructose	1.33	1.10	1.20

According to these investigators the influence of the sugar depends on its penetration, and the relative increase in respiration follows the order of penetrability. Similar results were obtained with the embryo of kidney bean. It is probable, however, that other factors than the degree of penetrability determine the respiratory values. The results of Maige and Nicolas do not agree with those obtained by Palladine, since in the former fructose has the fourth place in order of influence on respiration.

Molliard (1907) measured the respiration of aerial parts of radish which had been grown for about two months in cultures with and without sugar. The plants supplied with glucose and fructose showed a greater evolution of carbon dioxide per gram of dry matter per hour than did the plants grown in the culture medium without sugar.

Lubimenko (1906 a) investigated the influence of various sugars on the respiration of the plantlets of *Pinus Pinea* which were grown from embryos separated from the seeds. The plants were grown under sterile conditions.

It was found that saccharose, glucose, fructose, galactose, maltose, and lactose increased the amount of carbon dioxide evolved. Lactose was but slightly effective in this respect, while arabinose was apparently without any influence. The respiratory quotient was increased notably by saccharose, less by glucose or fructose, and only very slightly by the other sugars. In cultures supplied with either saccharose, glucose, or fructose, alcohol was formed as a product of respiration. It could be detected by its characteristic odor when the nutrient solution was distilled or the germinated embryos were heated.

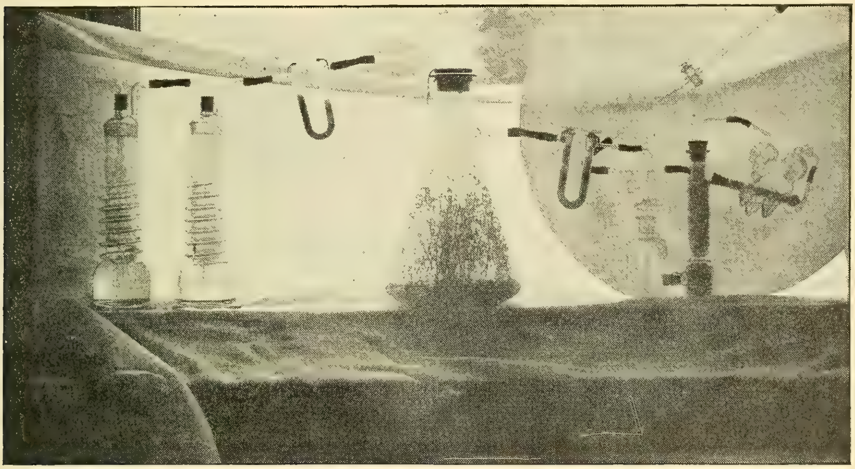

FIG. 7. RESPIRATION APPARATUS

It is clear, therefore, that sugar may be absorbed by leaves and shoots, and that the sugar absorbed increases respiration. The experiments just described, however, afford no information as to the continued effect of the sugars either on respiration or on growth. With the view of solving these two problems, and also of determining the period when the initial assimilation of the sugar occurs, the investigation here described was undertaken.

The growth chambers used in this experiment consisted of eight 2-liter suction flasks, shown in figure 8. Each culture chamber was fitted with a two-holed rubber stopper. Through one of the holes was inserted a thermometer, and through the other a glass tube extending to within a few inches of the bottom of the flask. The upper part of the glass tube was bent at a right angle, and attached to it was a U-tube filled with cotton. The side tube of the culture vessel was similarly provided with a U-tube. The culture chamber, with the U-tube, was connected on one side with a Friedrich gas-washing bottle containing a solution of potassium hydroxide (KOH) of a specific gravity of 1.27. This gas-washing bottle was in turn connected with a large 10-liter aspirator bottle, from which a large glass tube passed out of doors, whence the air was obtained. The side tube of the culture vessel was connected by means of the U-tube and glass and rubber tubing with a calcium chloride tower, which in turn was connected with a potash bulb having a small calcium chloride tube attached. Finally, the potash bulb was connected with a safety gas-washing bottle, and this bottle was connected with a Richard's pump.

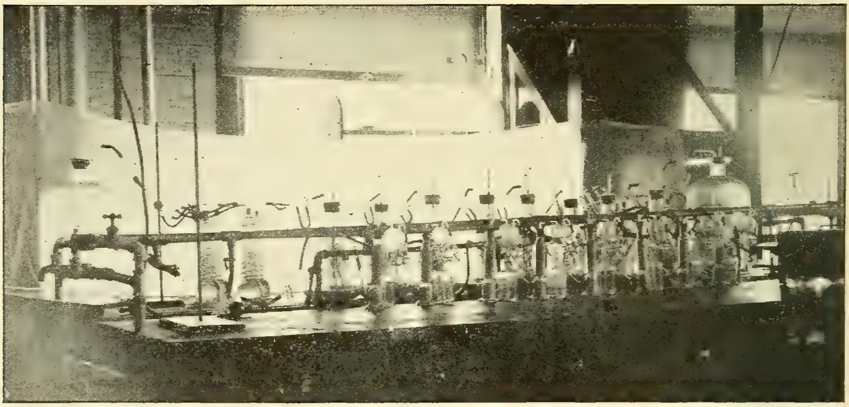

FIG. 8. RESPIRATION APPARATUS, SHOWING ARRANGEMENT OF WHOLE

All the usual technical precautions were observed in fitting the respiration apparatus, eight series of which were set up side by side. Two large 10-liter bottles were employed, to each of which four of the series were connected. The purpose of the large aspirator bottle was to permit the outside air to acquire the room temperature. The purpose of the cotton-filled U-tubes was to prevent access of spores or bacteria to the culture chamber. The entire apparatus is seen in figure 8, and an individual respiration apparatus in which two Friedrich bottles were used is shown in figure 7.

Pfeffer's solution was used, with the addition of 1.2 per cent of agar and the sugar to be tested. Control cultures lacking sugar were also set up. In each culture vessel was placed 300 cubic centimeters of the solution to be tested. The culture flask was then stoppered, and the entire top of the vessel swathed with cotton and cheesecloth to prevent the collection of spores or bacteria on the stoppers after sterilization of the vessels and their contents in the autoclave.

Eight separate lots of 100 seeds each, selected for uniformity, were placed in weighing dishes and weighed. The water content was also determined in sample lots. Into each of the weighing dishes, the capacity of which was 25 cubic centimeters, 20 cubic centimeters of the calcium hypochlorite solution was poured. The seeds were immersed for thirty-two hours, after which each lot was transferred to the culture vessels, the calcium hypochlorite solution being first poured off.

The seeds were sown on June 16 and the first respiration determinations were begun on June 17 at 11 a. m. The first weighings were made at 11 a. m. on June 18. Unfortunately the determinations for the first few days, except in a few cases, were not accurate. This was due to several causes — to leakage in some of the series, while in other cases the water pumps were not properly regulated. Beginning with June 20, however, the entire apparatus was in good working order, and the results from that date are consistent and accurate in so far as this type of apparatus will permit. It is seen in table 18, in which the results of the experiment are given, that by June 20 the sugar cultures showed greater evolution of carbon dioxide than did the check cultures. Individual cultures may show slight variation, but this is to be expected since a slight variation in growth, as regards both root and top development, may occur in the different growth chambers. The total carbon dioxide evolution in the duplicate cultures are in very close agreement.

The order in which the cultures are given in table 18 indicates the relative positions occupied by them in the experiment. Those at the right side of the table were slightly favored because of having a slightly better light exposure. This accounts for the greater increase in carbon dioxide of check culture 2 as compared with check culture 1, or of glucose culture 1 as compared with glucose culture 2. The maltose culture 2 became contaminated with a mold, and consequently the data are of little value except as a check on maltose culture 1 during the earlier stages of the experiment.

TABLE 18. INFLUENCE OF VARIOUS SUGARS ON RESPIRATION OF VETCH
(Figures in bold-faced type represent carbon dioxide evolved in the daytime)

Day and hour of examination	Carbon dioxide evolved (in milligrams)								Mean temperature (Centigrade)
	Maltose 1	Check 1 (no sugar)	Saccharose 2	Glucose 2	Saccharose 1	Check 2 (no sugar)	Maltose 2	Glucose 1	
June 17, 11 a. m., to June 18, 11 a. m..	......	......	......	......	14	12	......	......	21°
June 18, 11 a. m., to June 19, 11 a. m..	......	33	......	......	35	30	30	......	24°
June 19, 11 a. m., to June 20, 11 a. m..	......	......	......	41	36	31	34	39	21°
June 20, 11 a. m., to June 21, 11 a. m..	44	46	56	46	52	41	53	56	19°
June 21, 11 a. m., to June 22, 11 a. m..	68	61	66	52	77	51	87	78	20°
June 22, 11 a. m., to June 23, 10 a. m..	98	73	79	90	109	89	100	85	27°
June 23, 10 a. m., to June 24, 10 a. m..	129	105	126	117	123	94	137	121	30°
June 24, 10 a. m. to 5 p. m...........	**69**	**32**	**50**	**78**	**68**	**54**	**71**	**57**	30°
June 24, 5 p. m., to June 25, 10 a. m..	125	83	110	106	128	95	118	133	
June 25, 10 a. m. to 7 p. m...........	**31**	**34**	**58**	**63**	**54**	**46**	**88**	**75**	27°
June 25, 7 p. m., to June 26, 9 a. m..	70	53	77	70	76	51	99	85	
June 26, 9 a. m., to June 27, 10 a. m..	95	79	123	158	120	69	136	131	27°
June 27, 10 a. m. to 5 p. m...........	**19**	**21**	**39**	**32**	**27**	**18**	**30**	**22**	26°
June 27, 5 p. m., to June 28, 10 a. m..	84	59	86	78	84	56	119	103	
June 28, 10 a. m., to June 29, 10 a. m..	70	41	111	102	100	44	140	121	24°
June 29, 10 a. m. to 6 p. m...........	**12**	**10**	**34**	**22**	**6**	**12**	......	**18**	22°
June 29, 6 p. m., to June 30, 10 a. m..	47	30	69	57	62	31	......	66	
June 30, 10 a. m. to 4 p. m...........	**12**	**8**	**30**	**11**	**35**	**3**	......	**23**	22°
Total carbon dioxide evolved from June 20 to June 30............	964	735	1,117	1,082	1,121	754	......	1,174	....

The results as shown in table 18 indicate that as early as the fourth day the sugars were exerting an accelerative influence on respiration, and the relative differences between the sugar cultures and the checks increased with the progress of time. For example, on the fourth day the carbon dioxide evolution was 56 milligrams for saccharose culture 2 and only 41 milligrams for check culture 2; while during the last twenty-two hours of the experiment it was 99 milligrams for saccharose culture 2 as compared with 34 milligrams for check culture 2. In the early stages of growth the developing seeds had available the reserve food of the cotyledons. Toward the end of the experiment, however, the check plants were virtually starved, but the sugar-fed plants, constantly absorbing food from the medium, developed in an almost normal condition. The figures for carbon dioxide evolved during certain hours of daylight are shown in the table in bold-faced type. In nearly every case it will be noted that the carbon dioxide evolution in the daytime is greater in cultures supplied with sugar than in those lacking sugar. This is due,

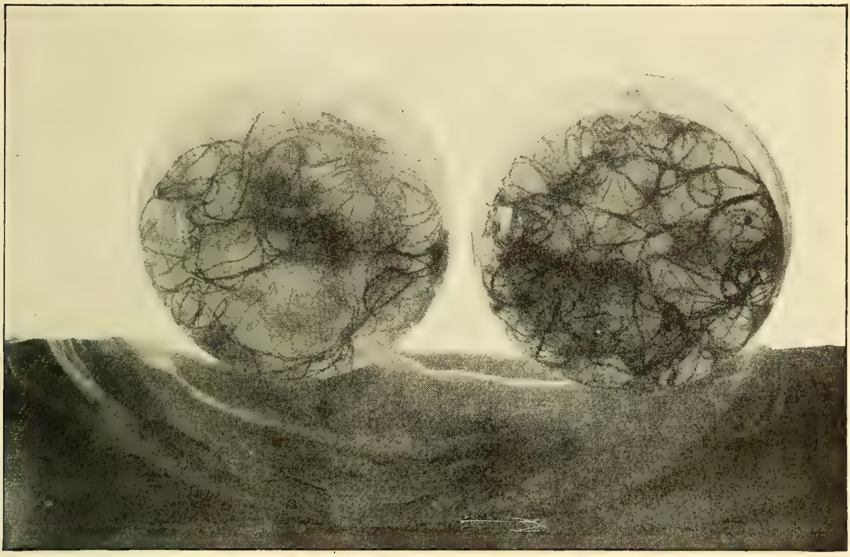

FIG. 9. INFLUENCE OF GLUCOSE ON ROOT GROWTH
Left, check (no sugar); right, glucose culture
(Photographed through bottom of culture vessel)

not only to an increased rate of respiration, but also to the fact that the root systems of the sugar-fed plants are greater than those of the check plants. This fact is brought out in table 19, and, in the case of the glucose and the check cultures, in figure 9.

All the data for this experiment are summarized in table 19. Since the respiration figures for the first three days were not obtained for all the cultures, it was necessary to make an estimate for these days in order to get an idea of the total carbon dioxide evolution. The estimated figure, given in the ninth column of the table, is 85 milligrams. This figure is based on the results obtained as given in table 18, and also on the results of a similar experiment in which the entire carbon dioxide evolution was determined. The figure is perhaps a little low, rather than high, and furthermore no distinction is made between the carbon dioxide evolved by the checks and that evolved by the sugar-containing cultures. The difference for this period, however, would be slight and would not at all affect the general conclusions.

An examination of table 19 reveals data of considerable interest. In general the dry weights of the roots of the sugar-fed plants are approximately double those of the check cultures. This is shown in figure 9 in the case of one of the glucose cultures. The weights of the tops in the various cultures show considerable similarity, although the sugar cultures average higher than the check cultures. It would appear that the behavior of the roots is very similar to that of a purely saprophytic organism.

It is of interest to note that the saccharose cultures and one of the glucose cultures showed a gain in weight over the original dry weight of the seed. The maltose and the glucose are not so beneficial as the saccharose. The sugar-fed cultures, as was to be expected, showed a greater growth than the checks, and a greater carbon dioxide evolution. The failure to lose in dry weight is not due to a conservation of the reserve food of the seed, but to the absorption of sugars, this absorbed food permitting greater growth, resulting in an increase in dry weight.

The glucose and starch equivalents of the carbon dioxide evolved are given in the last two columns. In the case of those cultures that showed a gain in weight, it would be logical to assume that the sugar absorbed equaled approximately the glucose equivalents. Unfortunately the sugar

TABLE 19. SUMMARY OF DATA CONCERNING INFLUENCE OF VARIOUS SUGARS ON GROWTH AND RESPIRATION OF VETCH

Culture solution		Original dry weight of seeds (grams)	Number of plants	Number of un-germinated seeds	Dry weight of roots (grams)	Dry weight of tops (grams)	Total dry weight (grams)	Gain or loss in weight (grams)	Carbon dioxide evolved (in grams)			Glucose equivalent to carbon dioxide (grams)	Starch equivalent to carbon dioxide (grams)
									June 17 to June 20	June 20 to June 30	Total		
Check (no sugar)	1	2.835	94	6	0.235	1.997	2.232	—0.603	0.085	0.735	0.820	0.5590	0.5031
	2	2.816	94	6	0.240	2.056	2.296	—0.520	0.085	0.754	0.839	0.5720	0.5148
Maltose, 2 per cent	1	2.820	94	6	0.447	2.224	2.671	—0.149	0.085	0.964	1.049	0.7205	0.6485
	2	2.832	95	5	0.476	2.288	2.764	—0.068	0.085	Contaminated toward end of experiment			
Glucose, 2 per cent	1	2.860	95	5	0.415	2.266	2.681	—0.179	0.085	1.175	1.260	0.8590	0.7731
	2	2.826	93	7	0.444	2.400	2.844	+0.018	0.085	1.082	1.167	0.7954	0.7159
Saccharose, 2 per cent	1	2.908	94	6	0.561	2.356	2.917	+0.009	0.085	1.121	1.206	0.8222	0.7400
	2	2.808	94	6	0.547	2.345	2.892	+0.084	0.085	1.117	1.202	0.8193	0.7375

determinations were not made. Qualitative tests showed the presence of reducing sugars in the media containing saccharose.

The results of the experiment are in agreement with the results obtained by Maige and Nicolas (1910) and contrary to the conclusion of Palladine (1901) — though it is hardly to be expected that the data from long-continued respiratory rates should agree with that for a short-period rate. Saccharose and glucose are somewhat similar in their beneficial effects, the former being slightly better than glucose, and glucose better than maltose.

Four attempts were made to repeat this experiment, but in every case the results had to be discarded owing to the occurrence of contaminations in nearly all the culture vessels. In every case the contamination was due to the introduction of one or two dead seeds into the growth chamber. It appeared to be almost impossible to sterilize the dead seeds, and no means was available for their detection; although it was noted in general that the failure of vetch seeds to bleach in the hypochlorite solution was indicative of failure to germinate. One preliminary experiment, in which glucose alone was tested in its effect on growth and respiration, yielded results similar to those obtained with that sugar in the experiment already described.

INFLUENCE OF VARIOUS CONCENTRATIONS OF SUGARS ON PLANT GROWTH

Molliard (1907) has shown that increasing the concentration of sugar beyond 2 per cent effects a corresponding increase in the dry weight of plants, although the higher concentrations do not permit of normal plant development. No investigations have been made on the influence of a series of lower concentrations of sugars on plant growth, and from the practical standpoint this is of greater importance; for it is evident that if organic matter is directly absorbed by a plant growing under normal conditions, then the plant must have the ability to take from the soil solution organic substances present in extremely weak concentrations. Aside from the practical aspect of the question, information on the subject is desirable for purely physiological reasons. Investigations were accordingly made with cabbage, sweet clover, crimson clover, and vetch.

EXPERIMENTS WITH CABBAGE (BRASSICA OLERACEA L.)

In the experiments with cabbage large test tubes were employed as culture vessels, each containing 50 cubic centimeters of the nutrient solution plus 1.2 per cent of agar and the sugar to be tested. A variety of cabbage known as Wakefield was used, and the seeds were carefully selected with regard to uniformity. The seeds were sterilized by immersion in the hypochlorite solution for sixteen hours. An attempt was made to sow in each tube twelve seeds, but this was not successful in all cases because of the haste necessary in sowing the seed so as to avoid danger of contamination. The experiment was started on December 20, 1913, and discontinued on February 18, 1914. The results with different concentrations of maltose are given in table 20:

TABLE 20. INFLUENCE OF VARIOUS CONCENTRATIONS OF MALTOSE ON GROWTH OF CABBAGE

(Plants grown in greenhouse. Duration, twenty-nine days)

Culture solution			Number of plants	Total dry weight (milligrams)	Average weight per plant (milligrams)	
Check (no sugar)		1	12	95	7.9	6.9
		2	11	63	5.7	
Maltose	0.1 per cent	1	13	98	7.5	7.7
		2	11	81	7.4	
		3	11	90	8.2	
	0.2 per cent	1	12	95	7.9	7.7
		2	12	86	7.2	
		3	12	96	8.0	
	0.4 per cent	1	12	112	9.3	9.0
		2	12	105	8.7	
	0.8 per cent		13	126	9.7	9.7
	1.5 per cent	1	12	126	10.5	11.5
		2	12	150	12.5	
	2.0 per cent	1	11	140	12.7	11.9
		2	12	134	11.2	
	2.5 per cent		12	174	14.5	14.5

The table shows a progressive increase in the dry weight of the cabbage with increase in concentration of maltose. At the lowest concentration there appears to be a slight increase in dry weight over the check, but the effect becomes marked only when a concentration of 0.4 per cent is reached.

In another experiment the influence of a mixture of saccharose and maltose was studied. The conditions of this experiment were identical with those of the preceding. The results, given in table 21, show that 2 per cent of maltose permits greater growth than that of the checks, and that the addition of saccharose increases the growth still further. Evidently the sugars are absorbed and assimilated.

TABLE 21. INFLUENCE OF A MIXTURE OF MALTOSE AND SACCHAROSE ON GROWTH OF CABBAGE

(Plants grown in greenhouse. Duration, twenty-nine days)

Culture solution		Number of plants	Total dry weight (milligrams)	Average weight per plant (milligrams)
Check (no sugar)	1	12	95	7.9
	2	11	63	5.7
Maltose, 2 per cent		12	134	11.2
Maltose, 2 per cent, plus saccharose, 0.4 per cent		12	153	12.7
Maltose, 2 per cent, plus saccharose, 1.6 per cent		12	168	14.0

In another experiment a culture containing 2 per cent of maltose plus 0.2 per cent of saccharose, with fourteen plants, yielded a dry weight of 138 milligrams, while a culture containing 2 per cent of maltose plus 1.6 per cent of saccharose, with the same number of plants, yielded a dry weight of 155 milligrams.

EXPERIMENT WITH SWEET CLOVER (MELILOTUS ALBA DESR.)

In the experiment with sweet clover the plants were grown in large test tubes, on a nutrient solution plus 1.2 per cent of agar. The seeds were sterilized by immersion for twenty-four hours in the hypochlorite solution. Seven seeds were sown in each test tube. Not every seed germinated, as is indicated in table 22, in which only the average results of the cultures are given. Unfortunately the results from the different cultures are not entirely comparable, owing to the difference in the number of plants per culture. Still this difference is sufficiently small to warrant the general conclusion that increase in concentration results in increased growth.

TABLE 22. INFLUENCE OF VARIOUS CONCENTRATIONS OF GLUCOSE AND SACCHAROSE ON GROWTH OF SWEET CLOVER

(Duration, June 19 to August 1, 1914, forty-three days)

Culture solution		Number of cultures	Total number of plants	Average number of plants per culture	Total dry weight (milligrams)	Average weight per plant (milligrams)
Check (no sugar)		18	83	4.6	708	8.5
Glucose	0.1 per cent	8	46	5.7	480	10.4
	0.2 per cent	6	29	4.8	318	11.0
	0.4 per cent	5	26	5.2	296	11.4
	0.8 per cent	5	28	5.6	328	11.7
	1.6 per cent	5	42	8.4	549	13.1
	3.2 per cent	5	15	3.0	346	23.1
Saccharose	0.1 per cent	9	54	6.0	547	10.1
	0.2 per cent	8	47	5.9	467	9.9
	0.8 per cent	6	29	4.8	479	16.5
	3.2 per cent	5	22	4.4	456	20.7

EXPERIMENT WITH CRIMSON CLOVER (TRIFOLIUM INCARNATUM L.)

In the experiment with crimson clover large test tubes were used as culture vessels, each containing 50 cubic centimeters of the culture solution plus 1.2 per cent of agar. The seeds were immersed in the hypochlorite solution for four hours. Maltose was the only sugar tested. The vigor

TABLE 23. INFLUENCE OF VARIOUS CONCENTRATIONS OF MALTOSE ON GROWTH OF CRIMSON CLOVER

(Duration, January 3 to February 21, 1914, forty-nine days)

Culture solution		Number of plants	Total dry weight (milligrams)	Average weight per plant milligrams)
Check (no sugar)	1	6	68	11.3
	2	5	68	13.6
Maltose	0.1 per cent	5	68	13.6
	0.2 per cent	5	77	15.4
	0.8 per cent	4	75	18.7
	1.5 per cent	5	85	17.0

of the plants varied directly with the concentration of the sugar, except in the case of the culture containing 0.8 per cent of maltose, in which the dry weight per plant is not in agreement with the normal results, due to the fact that only four plants were present in the culture. The results are given in table 23.

EXPERIMENT WITH VETCH

The experiment with vetch was conducted similarly to the preceding experiments. Unfortunately the cultures containing the lower concen-

TABLE 24. INFLUENCE OF VARIOUS CONCENTRATIONS OF MALTOSE AND OF LACTOSE ON GROWTH OF VETCH

(Duration, twenty-four days)

Culture solution	Plant	Length of tops (centimeters)	Length of roots (centimeters)	Total dry weight (milligrams)	Gain or loss in weight (milligrams)
Check (no sugar) 1	1	15.5	10.0	50	—10
	2	25.0	11.0		
Check (no sugar) 2	1	23.0	20.0	48	—12
	2	26.0	22.0		
Maltose 0.4 per cent	1	22.0	21.0	68	+ 8
	2	26.6	19.0		
Maltose 0.8 per cent	1	28.0	18.0	70	+10
	2	26.0	14.0		
Maltose 1.5 per cent 1	1	34.0	14.5	73	+13
	2	34.0	11.5		
Maltose 1.5 per cent 2	1	28.0	10.0	67	+ 7
	2	27.0	12.5		
Maltose 2.5 per cent	1	21.0	8.5	76	+16
	2	33.0	8.1		
Lactose 1 per cent 1	1	29.5	18.0	70	+10
	2	33.0	17.0		
Lactose 1 per cent 2	1	31.0	15.0	67	+ 7
	2	25.0	19.0		
Lactose 1 per cent 3	1	30.0	10.0	74	+14
	2	27.0	22.0		
Lactose 2 per cent 1	1	29.5	19.0	73	+13
	2	31.0	18.0		
Lactose 2 per cent 2	1	29.5	19.0	77	+17
	2	28.0	15.0		

trations of maltose had to be discarded, in the one case because of contamination and in the other case because only one plant developed. The results are given in table 24.

It is interesting to note that here again there is a marked effect on growth with increase in concentration, and furthermore that a concentration of maltose as low as 0.4 per cent is capable of influencing the yield of dry matter. It should be borne in mind, also, that the plants are in the seedling stage, when there has been available for them a considerable reserve supply from the cotyledons.

As was noted in many of the cultures, the addition of sugar gave rise to a production of color in the stalk. The check cultures, lacking sugar, produced plants with green stems, these being entirely devoid of the characteristic purplish pigment except at the base. The sugar-fed plants, on the other hand, were distinguished by the production of high color, and there was a gradation in intensity of color according to the concentration of the sugar.

ABSORPTION OF SUGAR FROM DILUTE SOLUTIONS

In the preceding experiments the lowest concentration employed was 0.1 per cent. As has already been stated, this is a relatively high concentration and one that is undoubtedly never attained in the soil solution. If the organic material in the soil is directly available to the higher plants, the substances must be absorbed from extremely dilute solutions, for the organic content of the soil solution is very small. But, while the concentration of the organic matter in the soil solution is extremely low, yet there must be a constant supply of soluble organic matter available to support the bacterial and the fungous flora.

The ability of the higher plants to absorb solutes from weak solutions has been amply proved for the nutrient and the non-nutrient salts as well as for dyes. All the nutrient salts are present in extremely dilute concentration in the soil solution. The mere fact that the amount of organic matter dissolved in the soil solution is extremely small is not a barrier to the utilization by the plant of the dissolved organic substances. The ability of vetch to remove glucose from a weak solution was tested by means of the following experiment:

Ten culture vessels were prepared, each containing 50 cubic centimeters of 0.0005 gram molecular glucose. Four of these were kept as checks, while in each of the other six were sown two seeds of vetch. The plants were grown for thirty-five days, and the solutions were then tested for glucose by means of Benedict's reagent. All the check solutions showed distinctly the presence of glucose; two of the culture solutions gave no tests whatsoever for glucose, three showed the merest trace, and the sixth, having a poorly developed plant, gave a fairly good indication of the presence of the sugar. The culture solutions were all tested for sterility by drop transfers to tubes of agar containing 0.5 per cent of glucose and Pfeffer's solution. In no case was any contamination shown. The culture solutions being free, therefore, from yeasts and bacteria, as well as from fungus mycelium, the conclusion is warranted that the glucose is absorbed by the vetch plant.

TOXICITY OF GALACTOSE FOR HIGHER PLANTS

In the course of these investigations it was noted (Knudson, 1915) that the sugar galactose was injurious to vetch, peas, corn, and wheat at a concentration as low as 0.0125 per cent. The injury was noted particularly in the roots. With higher concentrations the primary root tip coming in contact with agar containing galactose was killed, and lateral roots stimulated to formation met the same fate; so that ultimately a much-branched root was obtained. In case the entire primary root came into contact with the galactose-containing agar, it first assumed a brownish appearance and soon afterward died (Figs. 10 and 11). With Canada field pea it was noted that both lactose and raffinose increased growth. These two sugars, on hydrolysis, yield galactose as one of their products. It would appear, therefore, that galactose in the presence of glucose exerts no injurious effect. This was found to be the case. Glucose at a concentration of 1 per cent almost completely inhibited the toxic action of 1 per cent of galactose. In the previous experiments also it was noted that 1 per cent of galactose is antidoted by 1 per cent of glucose, but no other concentrations of glucose were employed. Accordingly an experiment was planned in which the galactose was to be maintained at a constant value and the concentrations of glucose were to be varied. Details of the experiment follow:

Large test tubes were used as culture vessels, each containing 33 cubic centimeters of the medium to be tested. The nutrient medium was

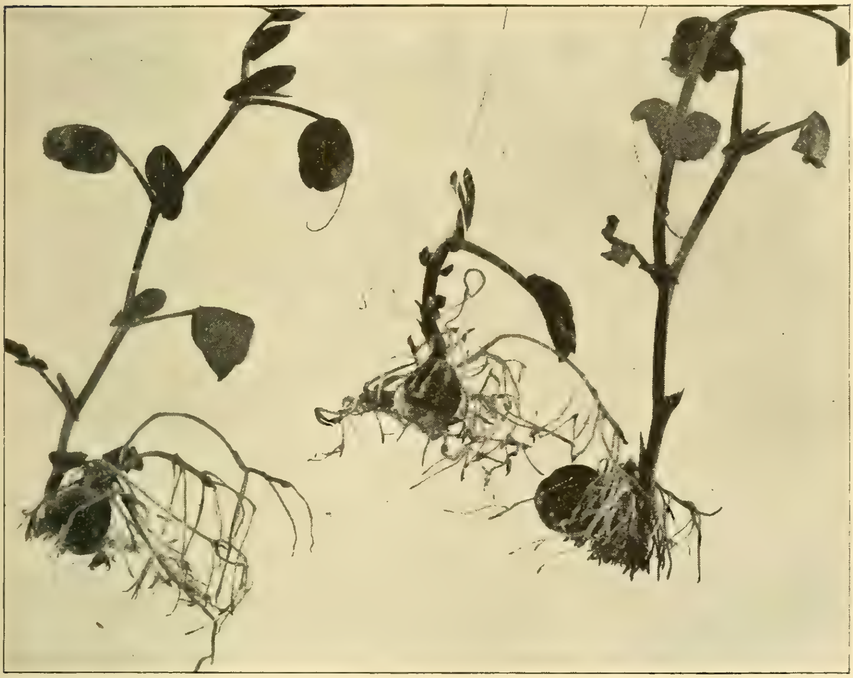

FIG. 10. TOXICITY OF GALACTOSE

All root tops were killed after contact with the agar medium containing galactose

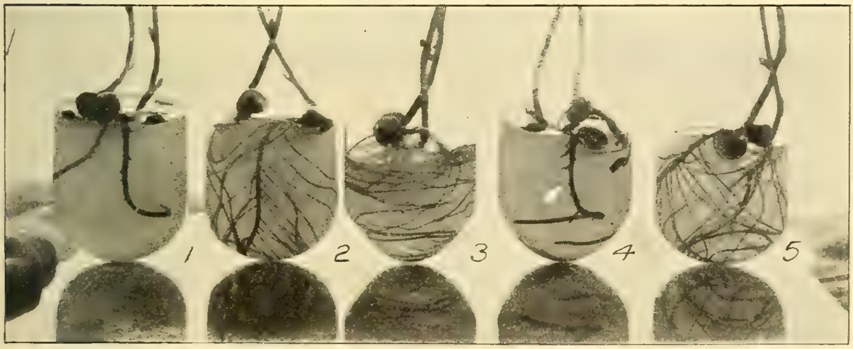

FIG. 11. ANTAGONISTIC ACTION OF GLUCOSE TOWARD GALACTOSE

1, Galactose, 0.025 mol.; 2, galactose, 0.025 mol., + 0.25 mol. glucose; 3, glucose, 0.05 mol.; 4, galactose, 0.025 mol., + 0.003125 mol. glucose; 5, check (no sugar)

Pfeffer's solution plus 1 per cent of agar, to which was added the sugar or sugars to be tested. Canada field pea was again employed and all cultures were made in triplicate. Two plants were grown in each culture. The plants were grown in the laboratory and not in direct light. The experiment was started on October 25, 1915, and completed on November 23, the duration being twenty-nine days. The concentration of galactose was constant, each of the cultures being of 0.05 molecular solution. The concentrations of glucose varied. The different combinations and the results are given in table 25, and other results are shown also in figure 11.

TABLE 25. ANTAGONISTIC ACTION OF GLUCOSE TOWARD GALACTOSE

(Duration, twenty-nine days)

Nutrient medium contains	Culture number	Appearance of roots	Average length of primary root (centimeters)
Galactose, 0.05 mol. Glucose, 0.20 mol.	1	Long, slender	9.0
	2	White, slender	9.0
	3	White, slender	13.0
Galactose, 0.05 mol. Glucose, 0.10 mol.	4	White, slender	9.0
	5	White, slender	9.0
	6	White, slender	10.0
Galactose, 0.05 mol. Glucose, 0.05 mol.	7	Slightly brownish	6.0
	8	Slightly brownish	7.0
	9	Slightly brownish	9.0
Galactose, 0.05 mol. Glucose, 0.025 mol.	10	Brownish	2.5
	11	Brownish, no penetration,* dead	
	12	Brownish, no penetration, dead	
Galactose, 0.05 mol. Glucose, 0.0125 mol.	13	No penetration of agar. All roots in contact with medium killed	
	14		
	15		
Galactose, 0.05 mol. Glucose, 0.00625 mol.	16		
	17		
	18		
Check (no sugar)	19	Long, slender, white	10.0
	20		9.0

* "No penetration" means that the roots do not grow into the agar.

The rather interesting result is noted that the antidotal influence of glucose is effectively attained only when the glucose is at a concentration greater than that of the galactose, although marked depoisoning action is effected when the concentration of glucose is equivalent to that of the galactose. When the glucose is still further decreased it is practically ineffective in exerting a protective action against the galactose. These results were confirmed by a second experiment similar to that just described. In the second experiment, however (Fig. 11), better antagonism resulted in the culture having equimolecular solutions of glucose and galactose.

This toxicity of galactose has been discussed before by the writer (Knudson, 1915) and, as there stated, no previous mention concerning it has come to the writer's notice. Molliard (1907) mentioned the non-availability of galactose for radish. Lubimenko (1906, a and b) reports no injury for *Pinus Pinea.* Meyer (1886), and later E. Laurent (1887), found that leaves and cuttings of certain plants placed in solutions of galactose absorbed the sugar, producing out of it starch. Most of the deamylated plants used by Meyer, however, did not produce starch when supplied with galactose, although glucose did cause starch production. It may be that the sugar itself is not toxic but that its oxidation products are injurious. The antagonistic action of glucose in this case might operate to render the cell impermeable to the galactose or alter the metabolic products of galactose. If the galactose, for example, could be transformed into starch or some other storage product — a possibility indicated by the work of Meyer and of E. Laurent — then its injurious action might be averted. The presence of a readily available sugar such as glucose might conceivably protect the galactose against oxidation and result in a transformation of the galactose into a storage product such as starch. It is of interest to note here that galactose may be utilized by many fungi and that it is fermented by certain yeasts. Further work on this interesting subject is now in progress.

SECRETION OF ENZYMES BY ROOTS

It has already been noted (page 12) that J. Laurent (1904) found inversion of saccharose when it was present in the culture medium, and this inversion was ascribed to the action of the invertase secreted by the roots of corn or of peas. Starch was likewise transformed, but this was ascribed to the action of diastase that had been secreted from the seed. Mazé (1899) reported a very marked inversion of saccharose in the culture medium when corn was employed, although in 1911 he reported contradictory results. Wohllebe (1911), investigating the secretion of diastase by roots, found a very weak secretion by the root hairs of corn, though in the other cases diastase secretion was effected by the disconnected root cap cells and by the dead root hairs. Czapek (1913) states that the evidence so far presented does not indicate any active ability on the part of the root to secrete enzymes.

The secretion of enzymes by plant roots is not within the scope of this paper. Investigations on this subject are now in progress and will be reported at a later date. Certain observations were made, however, tending to show that the enzyme invertase is secreted by roots of plants. The observations made are summarized separately in this place for the sake of convenience.

In a number of the experiments with vetch it was noted that in the saccharose cultures reducing sugars were found in the media at the conclusion of the experiment. In the water-culture experiments with vetch (page 39) the total amount of sugar remaining in two cultures at the end of the experiment was 0.422 gram and 0.413 gram, respectively. Of the former, 0.151 gram was reducing sugar and of the latter 0.125 gram was reducing sugar. In another experiment, with agar cultures (page 42), the total sugar remaining in each of two cultures was 3.544 grams, but in one the reducing sugar was 0.302 gram and in the other 0.342 gram. The culture media in all cases was in its reaction alkaline to methyl orange and acid to phenolphthalein. Somewhat similar results were obtained with radish and sunflower. There was very marked inversion of saccharose in the culture medium in which Canada field pea was grown, and some evidence was obtained on the excretion of raffinase (Knudson, 1915).

It has not yet been definitely proved that the inversion of saccharose is due to the invertase secreted into the culture medium. It is possible that the saccharose is inverted in the roots and the reducing sugars are secreted, but this is less probable. It is possible also that the enzyme may be released as a result of the death of root hairs or other cells of the roots, and that it is not secreted from living cells.

INFLUENCE OF SUGAR ON COLOR PRODUCTION

The work of Ewart, Overton, Wheldale, and others (Wheldale, 1911) indicates a close relationship between the sugar content of the plant and pigment production. It would naturally be expected, then, that the sugar supplied to plants would show production of anthocyan pigment, and this expectation has been realized. Throughout the experiments recorded in this paper a tendency to anthocyan production was noted, and in some cases brilliant red pigmentation was produced in the stems of vetch. In one experiment with vetch in which 0.05 molecular solution each of glucose and of maltose was used, at the end of two months the maltose-fed plants were colored throughout, the glucose-fed plants were colored to within half the distance of the top, and the control plants grown in the absence of sugar were entirely lacking in the pigment. The color disappeared in the course of a week on transferring the plants to the laboratory, where a diffuse light prevailed. The same loss of pigment was observed in other cases when the plants were transferred from the greenhouse to the laboratory. It was also noted that during periods of cloudy weather no pigment developed in plants grown in the greenhouse.

No marked difference was noted in the production of pigment by the different sugars. Lactose, however, appeared to be the least beneficial, although in one experiment with vetch this was not the case. The subject is to receive further attention.

GENERAL DISCUSSION

In a consideration of the increased yields of plants grown in the presence of sugars, it should be borne in mind that undoubtedly greater increase would have been attained had the plants been grown with their tops exposed to the atmosphere instead of being inclosed within a glass chamber. As already pointed out, the cotton plugs cause a retardation in the diffusion of carbon dioxide into the plant chambers. In the smaller tubes this is probably fully counterbalanced by the increased rate of production of carbon dioxide in the sugar-fed plants. The increased gain of these plants is due, therefore, not alone to the absorption and assimilation of sugar, but in part to their having a greater supply of carbon dioxide — produced, it is true, through the increased respiration of the plant — which carbon dioxide diffuses outward with difficulty owing to the presence of the cotton plugs.

The increases effected by the sugar, furthermore, are not so great as those obtained by Mazé (1889), by Mazé and Perrier (1904), and by J. Laurent (1904), because of the fact that the plants were grown entirely within the closed chamber. The atmosphere of the chamber was constantly of a high relative humidity, and consequently transpiration was retarded. The retardation of transpiration would of course decrease the absorption of sugar, since the sugar would not be rapidly removed from the zone of absorption owing to the lack of a rapidly ascending current of water. This would account partially also for the great influence of the sugar on root growth.

Special attention should be called to this very marked influence of saccharose, maltose, glucose, and fructose on root growth. In the presence of one of these sugars a very much-branched root system developed, the influence of the sugar on the root growth usually becoming evident within two weeks. Those plants grown in the absence of sugar, on the other hand, usually showed roots only slightly branched. It would appear that the sugar absorbed by the plant is largely utilized in the root itself, and perhaps there is but slight migration of the sugar to the stems and leaves of the plant. This would probably result in a lessening of the downward migration of the sugar, a consequence of which would be increased top growth. So very evident to the naked eye is the favorable influence of sugars on the root growth that one is inclined to consider the root as being partially saprophytic. (Fig. 6, page 43.)

Special attention should be called also to the ability of radish, vetch, and Canada field pea to utilize the sugar lactose. This sugar has not been found in the plant kingdom. The enzyme lactase has been found in but relatively few seeds of higher plants (Czapek, 1905), yet the sugar is used in demonstrable quantities and is apparently assimilated. This being the case, there must be a production of the enzyme lactase in response to the sugar lactose, and another case of the production of a qualitatively regulated enzyme may here occur.

Surprising also is the toxicity of the sugar galactose for the green plants when it is considered that it is utilized by various fungi and when its close similarity to glucose is recalled. Galactose differs structurally from glucose in that the positions of the O and OH radicals are reversed in the third asymmetric carbon.

Mazé and Perrier (1904) noted a loss of chlorophyll when glucose was supplied to corn, and Servettaz (1913) found the same condition for moss. These investigators consider that there is a loss of chlorophyll when its function is not necessary. It is more probable that the loss of chlorophyll is due to the changes resulting in the nutrient solution, as, for example, an unbalanced condition or the precipitation of the iron in hydrate form. The latter result has been noted in the Laboratory of Plant Physiology at Cornell.

Molliard (1907) believes that there is an antagonism between the absorption of sugar and chlorophyll function, with the result that injury is done to the plant. He states that there would be inverse currents of sugar, and, as he says, one can conceive that the two currents are injurious. Molliard's conclusions are based on two different series of experiments. In one series the plants were grown in tube cultures — in the first case with normal air, in the second case with 10 per cent of carbon dioxide, and in the third case with 10 per cent of carbon dioxide and glucose. The plant grown in 10 per cent of carbon dioxide gained 48 milligrams, while the plant grown in 10 per cent of carbon dioxide and glucose yielded only 32 milligrams dry weight; the control plant, growing without sugar and in normal air, yielded 16 milligrams dry weight. Molliard considers that the poorer growth of the glucose-fed plant is caused by an antagonism between the assimilation of carbon dioxide and the absorption of sugar. A more probable explanation is that the 10-per-cent carbon dioxide content is augmented by the increased respiration of the culture plants during the night, and that in the early stages of growth the air has constantly a higher concentration than 10 per cent of carbon dioxide and this increase may be injurious to the culture plant. The results of the experiments on respiration show that carbon dioxide is constantly eliminated during the daytime in the first sixteen days of growth.

In Molliard's second experiment the growth in open and in closed tubes was compared. Three plants were grown for two months in open tubes on 1-per-cent glucose. At the end of two months two of the tubes were sealed and the third was left open, but plugged with cotton. Six weeks later the weight of each plant was determined, with the following results:

	Fresh weight (milligrams)	Dry weight (milligrams)	Ratio of dry weight to fresh weight
Tube open	907	122	0.134
Tube closed	1,427	212	0.148
Tube closed	1,747	231	0.134

It will be noted that there was increased gain in the closed tubes. Molliard explains the increase here as due to the fact that the assimilation of carbon dioxide was prevented and consequently its injurious action was eliminated. A more probable explanation, in the writer's opinion, is that the plants in the closed tubes had a greater carbon dioxide content available than those in the cotton-plugged tube.

As was brought out in the respiration experiments with vetch, a constant elimination of carbon dioxide occurs in the sugar-containing cultures. This carbon dioxide is undoubtedly assimilated, and it is fair to assume that the carbon dioxide produced in respiration in darkness and in the constant respiration of roots affords a supply of greater concentration than that furnished by the normal atmosphere. The writer attempted experiments to test the contention of Molliard, but in each of the several experiments set up complications interfered with the results. In two experiments the plants grown in closed chambers with glucose supplied showed less gain than the plants grown in open chambers with air available. The plants were grown for only twenty days, however, and the experiments were necessarily stopped in each case because of the appearance of molds.

Lindet (1911) has stated that fructose induces tissue formation in plants, while glucose is utilized largely in respiration. He found that yeasts, *Penicillium glaucum*, *Aspergillus niger*, and the embryos of bean and barley, were influenced similarly by glucose and fructose. Glucose was more readily absorbed than fructose, but contributed largely to respiration. Fructose, on the other hand, in all cases increased the dry weights of the plants much more than did glucose. No cases similar to these have been noted in the writer's experiments, though the superiority of saccharose for vetch and peas may be explained by the work of Lindet. This subject of the rôle of glucose and fructose is now being intensively studied in the Laboratory of Plant Physiology at Cornell.

It has been demonstrated conclusively that various sugars can be absorbed by the roots of green plants and that these sugars are assimilated. Not only can sugars be absorbed and assimilated, but investigations show that methyl alcohol, glycerin, certain organic acids, and various organic nitrogenous and other organic substances, may likewise be utilized. The practical significance of these facts is immediately questioned.

Does the plant utilize substances from the soil, and, if so, what is their importance in the nutrition of the plant?

With respect to the absorption of humus and humate compounds, it has already been stated that J. Laurent (1904) and Mazé (1911) found such absorption. Molliard (1912), on the other hand, as a result of an interesting experiment, came to the conclusion that the humates of the soil are not absorbed. He grew plants under sterile conditions in a closed chamber on a loam rich in humus with no carbon dioxide supplied. The radish showed a slight increase in dry weight, due to the assimilation of carbon dioxide produced from decomposition of the soil humus. From his results Molliard concluded that none of the humus could have been absorbed.

The organic content of soils is of course extremely variable and few satisfactory data are available as regards the soluble organic material in the soil. According to Schreiner (1911) the organic content in ordinary soils is large. The average content of 237 types of United States soils, determined by analyses of thousands of samples, is 2.06 per cent for the topsoil and 0.83 per cent for the subsoil. In greenhouse practice the soils used have much higher organic content. In the forcing of cucumbers and tomatoes, for example, this organic content may be as high as 25 per cent or even higher.

As stated at the beginning of this paper, the problem of the relation of organic substances to plant nutrition is not merely that of the relation of humus. It is concerned with all the soluble organic substances that must arise from the decomposition of plant and animal residues. The practicability of the power of the plant to utilize to advantage various organic substances rests on the extent to which these substances are found in the soil and the ability of the plant to remove them from weak solutions.

There is found in most soils, then, a considerable quantity of organic material, but most of this is in an insoluble state and therefore nonavailable. It is constantly being acted upon, however, by enzymes secreted by microorganisms[3] and by other agents, and soluble organic substances are produced. The soluble organic substances are present in most soils in extremely low concentration, yet their sum total may be as high as or higher than the dissolved nutrients (Petermann, 1882). Gourley (1915) reports for certain orchard soils in New Hampshire a soluble organic content varying from ninety to two hundred and fifty parts per million. The fact that there exists in soils such a low concentration of soluble organic substances cannot be a serious argument against a direct nutritional value of the soil organic material. The mineral nutrients of the soil and the nitrates are probably present in concentrations no greater; yet these nutrients are constantly being absorbed, and the same is possible for the soluble organic substances, especially those that can be assimilated. The ability of vetch to remove glucose from weak solutions has been demonstrated, and, as stated at the beginning of this paper, the fact that microorganisms and saprophytic plants find in the soil their carbon requirements lends strength to the argument that the higher plants obtain, to their advantage, organic materials from the soil.

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[3] During the summer of 1913 a very marked stimulative effect of the fairy-ring fungus *Marasmius oreades* Fr. was noted on the growth of lawn grass on the Cornell campus, the grass in the region of the ring being darker in color and more vigorous in growth than that in other places. A possible explanation is the digestion of organic material in the soil by enzymes secreted by the fungus mycelium, and the utilization by the grass of the products of digestion.

Schreiner (1911) has called attention to the possibility of the favorable influence on plant growth of nitrogen and phosphorus containing organic compounds of the soil. He states: "The most beneficial manures under normal circumstances are those of organic origin, and the presence of such directly beneficial compounds, like creatinine, in well-rotted stable manure and in green manures, like cowpeas, goes far toward explaining why these manures are more beneficial to soil as a rule than are equivalent parts of fertilizer in the purely mineral forms." It has been demonstrated, however, that not only are organic nitrogenous substances available for plants, but carbohydrates, alcohols, and organic acids and their salts, can also be absorbed by the roots and assimilated by the plants.

In view, then, of the established ability of plants to absorb and assimilate organic substances, and in view of the presence in soils of insoluble organic substances which are constantly in a state of transformation to soluble organic compounds, it seems reasonable to conclude, with J. Laurent (1904), that "the organic matter of the soil plays a direct rôle in the nutrition of green plants independently of humus . . . . . in that the roots are able to find in the soil quantities of directly utilizable organic substances which in a weak measure contribute to the carbon nutrition of the plants."[4] It seems reasonable to conclude, furthermore, that under certain conditions, especially in greenhouse culture, the soil organic material may play a very important rôle in the organic nutrition of plants.

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[4] Translation from the original French.

SUMMARY

1. Corn (*Zea mays* L.) grown in nutrient solutions containing certain sugars is able to absorb these sugars by means of their roots, and the sugars are assimilated, effecting increased growth of the plant.

2. The sugars, in the order of their beneficial effect on the plant when grown in the light, are, first glucose and fructose, second saccharose, and third maltose. In the dark glucose again leads, while the other sugars are much alike.

3. The embryo of corn will develop in the absence of all endosperm material, and the presence of maltose increases growth. The production of pigment is progressively increased with the increase in concentration of glucose.

4. Canada field pea (*Pisum sativum* L.) responds in growth markedly to the presence of sugar; the sugars in the order of their beneficial influence being saccharose, glucose, maltose, and lactose.

5. Timothy utilizes glucose and saccharose, but not lactose when grown in the light. When grown in the dark lactose, as well as the other sugars, appear to be utilized.

6. Experiments with radish (*Raphanus sativus* L.) confirm the earlier investigations, glucose, saccharose, maltose, and lactose being utilized.

7. Vetch (*Vicia villosa* Roth) grown in the dark utilizes the various disaccharides, the order, as regards favorableness, being saccharose, maltose, and lactose. On vetch grown in the light the favorable influence of the different sugars is in the following order: saccharose, glucose, maltose, and lactose.

8. Data are herein presented showing the influence of sugar on the growth and respiration of vetch. The saccharose and glucose cultures are much alike in their effect during the period of the experiment; the maltose culture shows a lesser evolution of carbon dioxide. In the presence of saccharose the seedlings grown in the absence of carbon dioxide maintained practically the original weight of the seed, but in these experiments carbon dioxide equivalent to 0.822 gram of glucose in one case and to 0.8195 gram of glucose in another case was evolved. Somewhat similar results were obtained with glucose and with maltose.

9. The influence of sugar on respiration was manifest as early as the fifth day of the experiment.

10. The carbon dioxide evolution of the sugar-fed plants during the daytime is always appreciably greater than that of the check cultures. This is due in part to a greater root development in the sugar-fed cultures, but also to a greater rate of respiration.

11. Cabbage (*Brassica oleracea* L.) grown in the presence of maltose shows increased growth. The higher the concentration (2.5 per cent being the highest concentration employed), the greater is the yield of dry matter. A mixture of saccharose and maltose increased growth to a greater extent than 2 per cent of maltose alone.

12. Sweet clover (*Melilotus alba* Desr.) increases its growth with increased concentration of glucose or saccharose. Crimson clover (*Trifolium incarnatum* L.) behaves similarly when provided with maltose.

13. Vetch (*Vicia villosa* Roth) shows increased growth with increase in concentration of sugar.

14. Vetch (*Vicia villosa* Roth) is shown to absorb glucose from an extremely weak solution.

15. The sugar galactose is toxic to vetch, Canada field pea, corn, and wheat, even at concentrations as low as 0.0125 per cent.

16. The toxicity of 0.05 gram molecular galactose for Canada field pea is antidcted almost entirely by glucose when present at a concentration of 0.10 or 0.20 gram molecular. The toxicity of this solution of galactose is partially antidoted by 0.05 molecular glucose, but practically not at all antidoted when the glucose is less than 0.05 gram molecular.

17. The antagonistic action of glucose toward galactose may be due to its rendering the root impermeable to galactose. It is suggested that the toxicity of galactose may be due to its oxidation products, and that in the presence of glucose the metabolism of galactose may be altered.

18. Evidence was obtained indicating the inversion of saccharose by the roots of vetch, Canada field pea, radish, and sunflower. The inversion was due to invertase probably secreted from the roots. No secretion of maltose or lactose was noted.

19. In the presence of sugar there was noted a marked development of pigment in corn and in vetch.

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